Original article

A SYSTEMATIC REVIEW ON THE DIAGNOSTIC ACCURACY OF LINE PROBE ASSAY IN THE RAPID DIAGNOSIS OF DRUG RESISTANT TUBERCULOSIS IN INDIAN SCENARIO

Suzana B Shirly[1]; Reddy Kishore[2]; Bhargava Shipra[2]; Chauhan Manisha[2]

[1] Department of Microbiology, Christian Medical College, Vellore, India
[2] International Center of Excellence in Laboratory Training (ICELT)-FIND, National Tuberculosis Institute (NTI), Bangalore, India

DOI: http://doi.org/10.3126/saarctb.v19i1.39927
Received: 7th Jan Accepted: 5th February Published: 30thJuly
This article is available at: https://www.saarctb.org/stac-journal-2021/

ABSTRACT

Owing to the drastic increase in the number of patients with drug resistant TB around the world, it is important to increase the testing for it. Line probe assay (LPA) is the rapid diagnostic tool to detect drug resistant TB and it was endorsed by WHO for testing first line drugs such as Isoniazid (INH) and Rifampicin (RIF). This systematic review evaluated the accuracy of this LPA by analysing its sensitivity and specificity against the phenotypic drug susceptibility testing (DST) methods like LJ and liquid culture DST. A total of 4774 samples were included in this review from 19 articles. The average sensitivity and specificity for the detection of RIF resistance from 17 articles was 95.79% and 96.71% and for INH resistance it was 89.85% and 97.33% respectively when compared to phenotypic DST. Out of 19 articles included, 2 articles have mentioned the sensitivity and specificity for multi-drug resistant TB (MDR TB) and the average was 98.50 % and 97 % respectively. The accuracy for RIF resistance detection through first line LPA was good and the sensitivity detection for INH was less across the studies. This could be improved further in future generation assays. Our finding supports the use of LPA especially on smear positive specimens but use on smear negative specimens still be considered as studies have shown some interpretable results.

Key Words: Line Probe Assay LPA, Drug susceptibility testing (DST), DRTB, Isoniazid, Rifampicin

INTRODUCTION

Tuberculosis (TB) is a major cause of ill health and it's the leading cause of death from a single infectious agent. More than a quarter of the world's population is infected with M. tuberculosis which results in the development of TB disease. Around the world, an estimated 10 million people fell ill with TB in 2018. In India the number of patients diagnosed newly for TB varies from 1.2 million to 2.0 million between 2013 and 2018[(1)] .

The emergence of multidrug and extensively drug-resistant tuberculosis (MDR &XDR) is a major threat to global tuberculosis control. In 2018, there were about half a million new cases of rifampicin-resistant TB. Out of which 78% had multi drug resistant TB[(2)].Overall, there were an estimated 484,000 incident cases of MDR/RR-TB in 2018. Out of which 27% of MDR-TB cases had been reported from India. In addition to MDR TB cases a total of 13,068 cases of XDR-TB were reported in 2018[(1)].

Given the global statistics of drug resistant TB, it is important to increase the testing for drug resistance among bacteriologically confirmed TB cases and

Correspondence:
Ms. B. Shirly Suzana
Research Associate
Department of Microbiology
Christian Medical College
Vellore-3, India
E-mail:shirly0285@gmail.com
Mobile: +91 9566961955

it should be rapid. Due to which the number of patients having TB will be enrolled for treatment as early as possible.

The diagnosis of drug resistance of M.tuberculosis is done by performing drug susceptibility tests (DST) on clinical isolates either by using Lowenstein Jensen media or by automated liquid culture method such as Mycobacterium growth Indicator Tube (MGIT) system. These methods are laborious and they have longer turnaround time .Hence molecular methods were accompanied with conventional methods which detects the mutations in the genes responsible for drug resistance in M.tuberculosis.

The genotypic methods include line probe assays and nucleic acid amplification method like CBNAAT which reduce the time of detection from several weeks to few days[3]. Line probe assays such as , MTBDR plus and MTBDR sl (Hain life science) and the Cartridge Based Nucleic Acid Amplification tests (CBNAAT) like Xpert MTB/Rif assay have been endorsed by WHO for rapid and effective detection of M.tuberculosis as well as the genetic mutations in M. tuberculosis that confer drug resistance.

WHO has also endorsed the use of commercially available molecular line probe assays (LPA) like MTBDR plus and MTBDR sl (Hain life science) for rapid drug susceptibility testing of first-line drugs such as isoniazid and rifampicin as well as selected second-line drugs such as fluroquinolones and second-line injectable drugs only on smear positive pulmonary TB samples during the year 2008 and 2016 respectively. The turnaround time of this line probe assay is 1 to 2 days. The sensitivity and the specificity of the first line LPA was high with 98% and 99% compared to phenotypic methods with 87.7% and 89.7% respectively[4].

In India until the use of LPA for DST, conventional LJ and MGIT 960 were in practice. In 2011 first line LPA was included as part of programmatic management of drug resistant TB (PMDT) under National Tuberculosis Control Program (NTCP) by setting up LPA labs in several states of India starting with 30 labs which has extended to 64 labs till 2019. In 2011 the number of DST using LPA was 635 [5] which has substantially increased to 3,46,282 tests by 2019 [6], of which 10,837 MDR TB ,20,329 Isoniazid (INH) resistance and 2,247 rifampicin (RIF) resistance cases were identified.

In 2016 ,a systematic review was done on first line LPA with 74 publications by WHO from different part of the world including six publications from India[7]. The number of publications from India on LPA increases due to the drastic increase in the number of LPA tests performed in different parts of the country. A systematic review has been performed in order to elucidate the accuracy of LPA in Indian lab settings.

METHODOLOGY

We performed comprehensive search of the data bases like Pub med, Web of science and Google scholar for relevant citations. We have restricted to the time period from 2011 to 2019 as LPA became part of PMDT programme in 2011 in India. Key words used were TB, Drug resistant TB, Mycobacterium tuberculosis and accuracy of Line probe assay. We have included only full text articles and none from conference publications.

Studies published only on Indian data were included. The studies were prospective studies which compared LPA first line DST with a reference tests in a particular point of time. The reference standard tests were phenotypic tests like MGIT DST and LJ DST for first line drugs and their sensitivity and specificity data were included. Publications without sensitivity and specificity to detect both INH and RIF resistance, mono resistance to INH and RIF resistance were excluded. Studies with minimum 40 samples (both pulmonary and extra pulmonary samples) independent of the smear status were included.

The diagnostic accuracy of LPA first line DST with the sensitivity and specificity against the reference tests.

RESULTS

A total of 52 articles were collected which got published from different states of India on LPA and all these articles were full text articles. Of which 19 articles were included in this review as our systematic review focussed on the diagnostic

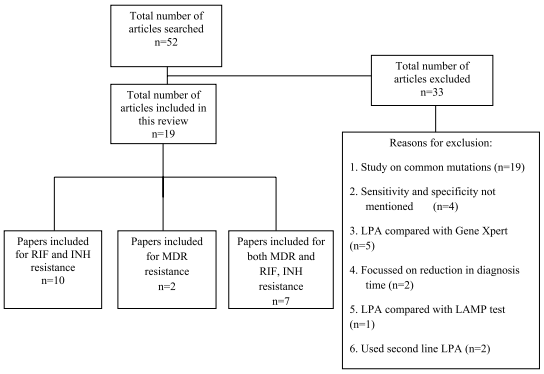

Figure 1. Characteristics of the studies included and excluded

accuracy of drug resistant tuberculosis using LPA compared to phenotypic diagnostic tests like LJ DST and liquid culture DST. Thirty three articles were excluded from this review and the reasons for not including these articles are mentioned in figure 1.

Table 1 demonstrates the characteristics of the 19 articles which provided data on RIF and INH resistance separately and also MDR TB (RIF and INH resistance together). All these 19 articles were prospective in design. Most of the studies were performed in either a regional or national reference laboratory setting.

Out of 19 articles, 15 evaluated LPA on direct sputum samples where 14 were on smear positive for Acid fast bacilli (AFB) and 1 was on smear negative samples .Four were on indirect samples/ culture isolates which are smear positive for AFB. Only eight studies have mentioned the version of the Hain genotype MTBDR *plus* kit used (version 1 (n= 3), version 2(n=5)).

Seven studies have mentioned the number of invalids in their study results. The reasons of these invalids were mentioned as incomplete amplification of RIF and/or INH genes or absence of TUB while the specimen is culture positive for *M.tuberculosis* in phenotypic test. None of the studies have mentioned indeterminate in their study results. Studies did not report whether repeat testing was done on the invalid results.

Table-1 -Characteristics of data on RIF and INH

S.No	Author	Year of publication	Reference Test	Source of the sample	Sample size	MDR (%)		RIF (Mono resistance) (%)		INH (Mono Resistance) (%)		Direct testing or Indirect testing	Design of the study
						Sens	Spec	Sens	Spec	Sens	Spec		
1	Binit Kumar Singh	2017	MGIT 960	Sputum (Sm -ve)	572	NA	NA	100	99.2	97.6	98.6	Direct testing	Prospective study
2	Richa Kumari	2016	LJ	EPTB	51	92.86	97.3	92.86	97.3	93.33	94.44	*M.tuberculosis* isolates	Prospective study
3	Raveendran	2012	Bact/Alert	Sputum (Sm+ve)	106	NA	NA	100	97	92	93	*M.tuberculosis* isolates	Prospective study
4	Shariq Ahamed	2017	LJ	Sputum (Sm+ve)	62	NA	NA	94.4	95.35	92	91.89	Direct testing	Prospective study
5	Raj N Yadhav	2013	LJ	Sputum (Sm+ve)	242	97	100	98	99	92	99	Direct testing	Prospective study
6	Neeraj Raizada	2014	LJ	Sputum (Sm+ve)	248	96	99	93	94	72	97	Direct testing	Prospective study
7	Jai shankar	2016	LJ	Sputum (Sm+ve)	105	100	94	NA	NA	NA	NA	Direct testing	Prospective study
8	Parveen Kumar	2013	MGIT 960	Sputum (Sm+ve)	567	93.1	100	96.8	100	92.8	100	*M.tuberculosis* isolates	Prospective study
9	Manoj Kumar	2016	LJ	Sputum (Sm+ve)	652	100	96	92	99	97	96	Direct testing	Prospective study
10	Jadhav	2015	LJ and MGIT	Sputum (Sm+ve)	47	95.74	100	NA	NA	NA	NA	Direct testing	Prospective study
11	Sana Nadrat	2017	LJ	Sputum (Sm+ve)	200	NA	NA	89	100	91	100	Direct testing	Prospective study
12	AK Mayura	2013	Bact/Alert	Sputum (Sm+ve)	125	97.7	99.1	95.8	98.5	96.3	98.4	*M.tuberculosis* isolates	Prospective study
13	Marilyn	2016	LJ/MGIT 960	Sputum (Sm+ve)	91	NA	NA	100	93.8	89.3	100	Direct testing	Prospective study
14	Madhuri	2015	LJ	Sputum (Sm+ve)	100	95	98.3	98.1	97.8	92.1	97.9	Direct testing	Prospective study
15	Pranali Medhaekar	2016	LJ	Sputum (Sm+ve)	510	98.9	97	98.9	96.8	97.5	98.4	Direct testing	Prospective study
16	Ritu Singhal	2012	MGIT 960	Sputum (Sm+ve)	120	NA	NA	97.6	94.4	83.3	93.8	Direct testing	Prospective study
17	Himanshu Vashishta	2017	MGIT 960	Sputum (Sm+ve)	483	NA	NA	87	87	76	97	Direct testing	Prospective study
18	Leimapokpam shivadutta	2014	Bact/Alert	Sputum (Sm+ve)	375	98.3	100	95	100	81.3	99.2	Direct testing	Prospective study
19	A.Jain	2016	MGIT 960	Sputum (Sm+ve)	118	NA	NA	100	95	92	100	Direct testing	Prospective study

Outcome of Interest:

Table 2. Average Sensitivity and Specificity of RIF, INH and MDR TB detection of first line LPA compared to phenotypic tests.

	Sensitivity	Specificity
RIF resistance detection	95.6% (87-100)	96.25% (87-100)
INH resistance detection	88.70% (72-100)	97.59 % (91.89 -100)
MDR TB detection	98.50% (92.86-100)	97% (94-100)

A total of 4774 samples were included in this review from 19 articles. As shown in table 2 the average sensitivity and specificity for the detection of RIF resistance was 95.79% (ranges from 87% to 100%) and 96.71% (ranges from 87% to 100%) and for INH resistance it was 89.85% (ranges from 72% to100%) and 97.33% (ranges from 91.89 % to 100%) respectively. Out of 19 articles included, 2 articles have mentioned the sensitivity and specificity of MDR TB detection (RIF and INH resistance together) and the average was 98.50 % (ranges from 92.86 % to 100%) and 97 % (ranges from 94 % to 100%) respectively.

Diagnostic accuracy of LPA from Direct testing compared to phenotypic tests:

A total of 3925 samples from 15 studies were tested on LPA directly from specimens which showed the average sensitivity and specificity as 95.61% and 96.25 % for RIF resistance, 88.70% and 97.59% for INH respectively. Two articles have mentioned the sensitivity and specificity of MDR TB detection (RIF and INH resistance together) for which the average sensitivity and specificity was 97.25 % and 97.67% respectively.

Diagnostic accuracy of LPA from Indirect testing compared to phenotypic tests:

A total of 4 articles performed LPA on culture isolates with the sample size of 849. The average sensitivity and specificity of RIF resistance detection was 96.36% and 98.20% .For INH resistance the sensitivity and specificity were 93.60% and 96.46% respectively.

Hain genotype MTBDR plus Version 1 vs. Version 2:

Eight articles have mentioned the version of kit used. The average sensitivity and specificity of RIF resistance detection using Version 1, Version 2 was 94.78%, 98.40% and 94.56%, 99.60% respectively. Similarly the average sensitivity and specificity of INH resistance detection was 83.76%, 95.20% and 98.32%, 99.30% respectively.

DISCUSSION

This literature search identified 17 articles which reported the sensitivity and the specificity of RIF and INH detection. Two articles reported the sensitivity and specificity of MDR TB detection. Higher sensitivity and specificity was observed for RIF and INH detection such as 95.61% and 96.25 % for RIF resistance, 88.70% and 97.59% for INH respectively. Raizada et al from India has also reported high sensitivity and specificity for RIF and INH detection which similar to our findings[8].

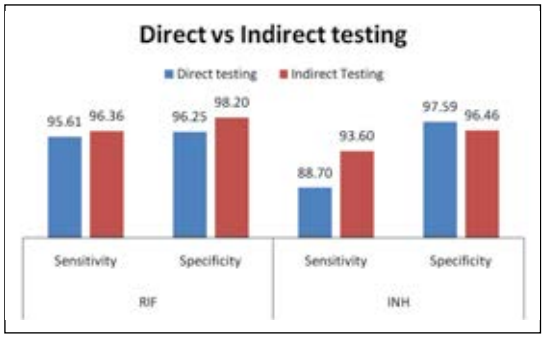

Figure 2 Sensitivity of LPA for indirect testing

On the other hand when sensitivity of RIF resistance detection was compared with the INH found that sensitivity of INH detection was less. The overall sensitivity for RIF detection was 95.79% and for INH resistance detection it was 89.85 % only. Similarly Singhal et al [9],Ninan et al from India[10] and Maschmann et al from Brazil [11]have reported less sensitivity and secificity for INH detection in their study.

According to Barnard et al in 2008 the low sensitivity range in the detection of INH resistance is due to the mutations being detected in a wide range of genetic loci compared to RIF[12]. In addition Meaza et al in 2017 have stated that nearly 10-25 % of INH resistant strains have mutations outside kat G and inh A regions[13].

As shown in figure 2 sensitivity of LPA for indirect testing was higher for both RIF and INH detection when compared to direct testing and no studies performed LPA testing on specimens and culture isolates from the same patients. This might be due to the increased bacillary load when using culture isolates for LPA compared to direct samples in the studies included in this review. This study finding was in contrast with the finding from Nathavitharana et al in 2016 where they have reported less sensitivity and high specificity in indirect testing compared to direct testing [14].

There was no significant observation on smear data in this review. However our review demonstrated that assay performed well in smear positive samples as invalids from the included studies were mainly smear negative specimens which was similar to the findings of Meaza et al in 2017[13] , Yadhav et al in 2013[15]and Ahmed et al in 2017[16] . Binit Kumar Singh et al in 2017 has reported high sensitivity and specificity for RIF and INH resistance detection in LPA on sputum smear negative pulmonary TB cases in his study[17]. Further studies are needed which compares the accuracy of LPA on smear negative with smear positive samples.

We performed a comprehensive search of articles through different databases. Review of the articles was done independently. The quality review of the studies and disagreements were sorted out with discussions. This review was limited by small numbers of available studies on first line LPA published only in India. Most of the studies from India focussed on the mutations associated with the RIF and INH drug resistant TB. We can foresee more publications towards the clinical impact of LPA like patient management and treatment outcomes and how much LPA has been contributed in the rapid diagnosis over conventional tests.

CONCLUSION

We have observed excellent accuracy for RIF resistance detection through first line LPA. As RIF resistance is the surrogative marker for MDR TB, LPA can serve as a good diagnostic tool for MDR TB detection in high TB burden countries like India. As the sensitivity detection for INH was less across the studies, it could be improved further in future generation assays. Our finding supports the use of LPA especially on smear positive specimens but use on smear negative specimens still be considered as studies have shown some interpretable results. With good microbiological laboratory practices there is a high chance of improving the quality of testing with minimal invalids or indeterminate results as they are the mainly due to the mistakes during setup or performance of the amplification reaction or presence of amplification inhibitors.

CONFLICT OF INTEREST

None

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