Int.J.Curr.Microbiol.App.Sci (2024) *13*(02): 115-118

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 2 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1302.016

Burden of Isoniazid Monoresistant Tuberculosis in Sputum Sample of Patients Attending a Tertiary Care Center, Trichy, India

S. Senthil Vaishaini, P. Gnanaguru and K. Lakshmi

Department of Microbiology, KAPV Govt. Medical College, Trichy, Tamil Nadu, India

**Corresponding author*

Keywords

Tuberculosis, morbidity, mortality, Mycobacterium, hybridization technique

Article Info

Received:
15 December 2023
Accepted:
22 January 2024
Available Online:
10 February 2024

A B S T R A C T

In drug resistant tuberculosis (DRTB), Rifampicin resistance has always been prioritized hence Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is recommended. However, since it doesn't detect isoniazid resistance, rifampicin sensitive patients with unknown isoniazid status may be erroneously treated as drug sensitive TB leading to poor treatment outcomes and emergence of multidrug resistant (MDR) TB. Hence this study was undertaken to estimate the prevalence of isoniazid monoresistant TB and to find out the level of isoniazid resistance using Line probe assay (LPA). A total of 3227 consecutive sputum samples of patients between July 2023 and December 2023 were enrolled for the study. The samples were subjected to smear microscopy, CBNAAT and LPA (Line Probe Assay) and the results were documented. In our study the prevalence of isoniazid monoresistance was estimated to be 8.2%. Among isoniazid monoresistance the prevalence of high level resistance was 65.7% and that of low level resistance was 31.5%. This shows that isoniazid monoresistance forms a major chunk of DRTB, with majority of patients having high level resistance to isoniazid. Thus, LPA serves as a rapid diagnostic tool especially for the detection of isoniazid monoresistance.

Introduction

Tuberculosis has earned the sobriquet "The Captain Of All These Men Of Death" during the 18th and 19th century owing to its morbidity and mortality and it continues to be a global crisis. Despite being a curable disease it ranks eighth among the leading cause of death (WHO, 2011-2015). Multidrug resistant tuberculosis defined as the resistance of Mycobacterium tuberculosis to two of the first line drugs rifampicin and isoniazid has plagued the treatment of Tb over the past two decades (Chowdhury *et al.,* 2023). Significant setbacks in the management of resistant tuberculosis are the lack of a rapid and accurate diagnostic modality, cost of treatment, and adherence to treatment. Rising rates of drug-resistant tuberculosis in India necessitate a rapid, low-cost, robust test to detect the same.

Molecular techniques have revolutionized the diagnosis of tuberculosis – both susceptible and resistant. CBNAAT and LPA are a part of the standard of care that is now used under the programmatic management of drug-resistant tuberculosis (PMDT) (WHO, 2012) under NTEP. Genotype MTBDRplus assay (LPA) is a reverse

hybridization technique which targets the rpoB (coding for beta subunit of RNA polymerase), katG (coding for catalase peroxidase) and promoter region of inhA (coding for NADH enoyl ACP reductase) genes in both culture and clinical samples. Thus, in addition to detecting rifampicin resistance it also detects high level and low level resistance to isoniazid via katG and inhA gene respectively. This study aims at finding out the prevalence of isoniazid monoresistance and the level of resistance using LPA.

Materials and Methods

Study Setting

Microbiology department K.A.P.V Government Medical College, Trichy. The mycobacteriology laboratory here is an accredited center for culture and drug susceptibility testing and it performs CBNAAT and LPA as a part of Programmatic Management of Drug Resistant Tuberculosis.

Participants

Following approval from the Institutional Ethics Committee sputum samples of suspect TB cases who fit the inclusion and exclusion criteria (refer Table 1) were collected after informed consent. The samples were first subjected to smear microscopy followed by CBNAAT and LPA.

Specimen Processing

All CBNAAT positive, smear positive sputum samples were directly subjected to LPA by decontaminating the sample followed by extraction and amplification of DNA and finally hybridization of the amplified DNA on membrane strips coated with complementary probes targeted against specific genes.

All CBNAAT positive, smear negative samples were processed through liquid culture in Mycobacterial growth indicator tube preceding LPA. Growth on MGIT tube was examined microscopically for acid fast bacilli and confirmation of Mycobacterium tuberculosis was done with an immunochromatographic test. Only after this these samples were subjected to LPA.

A valid LPA result was defined by a Mycobacterium tuberculosis complex-specific control (TUB), conjugate controls (CC) and amplification control (AC) bands in conjunction with the target gene locus control. The presence of wild type band without a mutant band is interpreted as resistance not detected and the presence of mutant band with presence of its corresponding wild type band is interpreted as resistance detected.

A summary of test results and corresponding clinical interpretation of LPA is provided in Table 2.

Results and Discussion

A total of 3227 sputum samples were collected of which 1333 were both CBNAAT and smear positive and 92 samples smear negative and CNAAT positive.

Around 1425 sputum samples were analysed using LPA of which 40 samples were excluded as they showed no TUB bands (Figure 1). Of the remaining 1385 samples which were analysed 30(2.1%) showed MDR pattern (Figure 2), 23(1.6%) showed Rifampicin monoresistant pattern (Figure 3) and 114(8.2%) showed Isoniazid monoresistant pattern. Of the 114 Isoniazid monoresistant sample 75(65.7%) showed mutation in katG gene (Figure 4), 36(31.55%) showed mutation in inhA gene (Figure 5) and 3(2.6%) showed mutation in both katG and inhA gene (Figure 6).

In India, the number of people with tuberculosis amounts to a fifth of cases seen globally. As per the Global TB Report 2021, the estimated incidence of all forms of TB in India was 188 per 100,000 population (WHO, 2022).

For several decades, ZN smear microscopy has been the mainstay in the diagnosis of PTB, particularly in resource-limited, high-TB-burden countries such as India (Aber *et al.,* 1980). However, the technique has low sensitivity, is highly observer-dependent, and is incapable of distinguishing between MTBC and NTM strains (WHO, 2011). Hence WHO recommends the use of approved rapid molecular test like CBNAAT and LPA as the initial test to detect TB disease as well as resistance to several anti-TB agents before initiating therapy (WHO, 2022).

Apart from its rapidity LPA can also provide information on the mutation pattern which can influence treatment. If inhA is the only mutation present it is likely that isoniazid can still be effective at a higher dose whereas if katG mutation is present isoniazid is no longer effective even at a high dose.

According to The National Strategic Plan for Elimination of Tuberculosis 2017-25 with the goal of Ending TB by 2025 the End TB targets for India is driven by the DETECT - TREAT - PREVENT - BUILD approach (India tuberculosis annual report, 2022). The focus is on early diagnosis of all the TB patients, prompt treatment with the right drugs and regimens along with suitable patient support systems including financial and nutritional support (India tuberculosis annual report, 2022). This study was conducted to assess the prevalence of isoniazid monoresistant Tb using LPA. Our study population showed the prevalence of isoniazid monoresistance to be 8.2% whereas a prevalence rate of 1-7% have been observed in various other Indian studies (D'souza *et al.,* 2009; Sharma *et al.,* 2014; Yacoob *et al.,* 2016). Our study population showed a higher prevalence of katG mutation when compared to that of inhA mutation similar to other studies.

Table.1 Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
All CBNAAT positive sputum samples.	All extrapulmonary samples.

Table.2 Clinical interpretation of FL-LPA (first line line probe assay)

Drug	Gene	Results	Clinical Interpretation
Rifampicin	RpoB	Resistance inferred or detected	R is not effective.
Isoniazid	katG	Resistance inferred or detected	H is unlikely to be effective even at high doses.
	InhA	Resistance inferred or detected	H at high doses is likely to be effective.

Table.3 Prevalence and pattern of drug resistance by LPA.

Type of Mutation	No. of Samples	Percentage
KatG	75	65.7%
InhA	36	31.5%
Both	3	2.6%

Table.4 Prevalence and pattern of mutation causing isoniazid resistance observed with LPA.

Pattern of Resistance	No. of Samples	Perecentage
MDR (resistant to rifampicin and isoniazid)	30	2.1%
Rifampicin monoresistant	23	1.6%
Isoniazid monoresistant	114	8.2%

The prevalence rate of MDR in our study was 2.1% which correlated with the study of Sharma *et al.,* (2014). Our study shows that the major burden of drug resistant Tb is attributed to isoniazid monoresistance especially high - level resistance.

Hence prompt detection of drug resistant pattern of Tb using molecular methods like LPA may prove game changing in the management of drug resistant TB.

Author Contribution

S. Senthil Vaishaini: Investigation, formal analysis, writing—original draft. P. Gnanaguru: Validation, methodology, writing—reviewing. K. Lakshmi:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Conflict of Interest: The authors declare no competing interests.

References

Aber V R, Allen B W, Mitchison D A, Ayuma P, Edwards E A, Keyes A B. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. Tubercle. 1980 Sep 1;61(3):123-33. https://doi.org/10.1016/0041-3879(80)90001-x

Chowdhury K, Ahmad R, Sinha S, Dutta S, Haque M. Multidrug-Resistant TB (MDR-TB) and Extensively Drug-Resistant TB (XDR-TB) Among Children: Where We Stand Now. Cureus. 2023 Feb 18;15(2). https://doi.org/10.7759/cureus.35154

D'souza D T, Mistry N F, Vira T S, Dholakia Y, Hoffner S, Pasvol G, Nicol M, Wilkinson R J. High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the Revised National Tuberculosis Control Programme in an urban metropolis (Mumbai) in Western India. BMC Public Health. 2009 Dec;9(1):1-9. https://doi.org/10.1186/1471-2458-9-211

Global Tuberculosis Report 2022 by World Health Organization. Journal of Diagnostics Concepts & Practice.

India tuberculosis annual report 2022.

Sharma S, Madan M, Agrawal C, Asthana A K. Genotype MTBDR plus assay for molecular detection of rifampicin and isoniazid resistance in Mycobacterium tuberculosis. Indian Journal of Pathology and Microbiology. 2014 Jul 1;57(3):423-6. https://doi.org/10.4103/0377-4929.138738

World Health Organization. Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis: policy statement. World Health Organization; 2011.

World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis: emergency update 2008. World Health Organization; 2012.

World Health Organization. The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis.

Yacoob F L, Philomina Jose B, Karunakaran Lelitha S D, Sreenivasan S. Primary multidrug resistant tuberculosis and utility of line probe assay for its detection in smear-positive sputum samples in a tertiary care hospital in South India. Journal of Pathogens. 2016 Mar 23;2016. https://doi.org/10.1155/2016/6235618

How to cite this article:

Senthil Vaishaini, S., P. Gnanaguru and Lakshmi, K. 2024. Burden of Isoniazid Monoresistant Tuberculosis in Sputum Sample of Patients Attending a Tertiary Care Center, Trichy, India. *Int.J.Curr.Microbiol.App.Sci.* 13(2): 115-118. **doi:** https://doi.org/10.20546/ijcmas.2024.1302.016