International Journal of Research in Medical Sciences
Dhapalapur NR et al. Int J Res Med Sci. 2025 Apr;13(4):1499-1503
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20250972

Original Research Article

Detection of pyrazinamide resistance among multidrug resistant tuberculosis in North Karnataka

Nivedita R. Dhapalapur*, Nirmala, Archana Hegde M.

[1]Department of Microbiology, JSS Medical College and Hospital, Mysuru, Karnataka, India
[2]Senior Specialist, STDC/IRL, Ministry of Health and Family Welfare Office, Government of Karnataka, India
[3]Scientist, Devigere BioSolutions, Pvt Ltd, Bangalore, Karnataka, India

Received: 31 January 2025
Revised: 06 March 2025
Accepted: 12 March 2025

***Correspondence:**
Dr. Nivedita R. Dhapalapur,
E-mail: nivurd1996@gmail.com

ABSTRACT

Background: Pyrazinamide was identified as a result of a structural activity connection with nicotinamide, which exhibits antitubercular properties (PZA). PZA possesses the exceptional capacity to be directly identified in vivo, first in *Mycobacterium TB*-infected mice and guinea pigs and subsequently in clinical settings. Pyrazinamide (PZA) is a newly developed antituberculosis (anti-TB) medication that is necessary to shorten the duration of TB treatment. Drug-resistant and drug-susceptible TB, including multidrug-resistant TB, require PZA as part of any therapy regimen because it eliminates nonreplicating persisters that other TB treatments are unable to.
Methods: All clinical specimens presented to the centre for follow-up or for newly diagnosed cases requiring programmatic treatment of DRTB were included in the research. Salivary or insufficient samples, as well as samples that satisfied the centre's SOP rejection criteria, were excluded from the research. MGIT-based susceptibility testing was used to evaluate the PZA susceptibility for 609 isolates.
Results: Of the 11104 samples, 207 (1.8%) were identified as MDR, 101 (0.9%) as mono-rifampicin resistant, and 619 (5.5%) as mono-isoniazid resistant. The BACTECTM MGITTM 960 technique was then used to cultivate 609 samples. The Bioline TB Ag MPT64 test is a quick way to find the M. tuberculosis complex. PZA susceptibility for 609 isolates was assessed using MGIT-based susceptibility testing. PZA resistance was detected in 194 (31.8%) of the 609 isolates.
Conclusions: The results may emphasise the necessity of regular DST for PZA in Indian public health labs and more study to better identify the variables linked to PZA resistance. In order to inform evidence-based approaches for tuberculosis control and care, this study also highlights the significance of ongoing surveillance of medication resistance patterns.

Keywords: DST, MDR, North Karnataka, Pyrazinamide

INTRODUCTION

A structural activity relationship with nicotinamide, which has antitubercular effects, led to the identification of pyrazinamide (PZA).[1] PZA has the unique ability to be directly detected in vivo, initially in mice and guinea pigs infected with *Mycobacterium TB* and then in clinical cases.[2] A novel antituberculosis (anti-TB) drug called pyrazinamide (PZA) is essential for reducing the duration of TB treatment. PZA is a necessary component of any drugs combination used to treat drug-susceptible and drug-resistant TB, including multidrug-resistant TB, because it eliminates nonreplicating persisters that other TB medications are unable to eliminate.[3]

The pncA-encoded mycobacterial pyrazinamidase (PZase) activates the prodrug pyrazinamide (PZA). Pyrazinoic acid, the resulting active product, has the ability to sterilise *Mycobacterium tuberculosis* complex (MTBC) infections.[4] PZA is vital for the treatment of MDR-TB as

well as drug-susceptible TB. Its special ability to sterilise persister bacilli has been shown to reduce the length of treatment for drug-susceptible tuberculosis from 12 months to 6 months.[5,6]

Multidrug-resistant tuberculosis (MDR-TB) remains a significant public health issue in India.[8,9] According to the 2020 Global TB Report, roughly 10 million individuals contracted TB in 2019, of whom half a million acquired rifampicin-resistant TB, and 78% were diagnosed with multidrug-resistant TB. Therefore, resistance to first- or second-line anti-TB medications is a significant factor in TB morbidity and death.[10] Statistics from the World Health Organisation (WHO) from 2018 show that there were around 0.48 million new cases of rifampicin-resistant TB worldwide, with multidrug-resistant TB accounting for 78% of these cases.[9]

The World Health Organisation (WHO) recommends PZA for MDR-TB treatment plans as well. PZA inclusion may enhance the effectiveness of MDR-TB treatment in PZA-susceptible MDR-TB. PZA is crucial for treating MDR-TB current and in the future due to possible synergistic effects with key second-line medications and new medications (such bedaquiline, delamanid, and pretomanid).[7]

PZA susceptibility testing require an acidic environment since PZA only exhibits antibacterial action at low pH values. Only a small number of labs do PZA susceptibility tests because of the intricate processes and high failure rate. Nevertheless, combined research indicates that 16.2% of TB patients have PZA resistance. PZA resistance rates in MDR-TB patients range from 36 to 85%, while in non-MDR-TB patients they range from 2 to 7.5%. Thus, accurate PZA resistance prediction before to therapy aids in the development of more potent therapies.[11]

PZA resistance may result from mechanisms other than pncA mutations. For instance, PZA resistance may result from changes to the POA binding site caused by mutations in rpsA, which codes for 30S ribosomal S1 proteins.[13] According to certain research, panD is linked to PZA resistance.[11] More experimental evidence is required to elucidate the roles of rpsA and panD mutations to PZA resistance, as researchers continue to debate their significance in PZA resistance.[11]

It is truly challenging to undertake phenotypic PZA drug susceptibility testing (DST) as part of routine care or drug surveillance. Because PZA needs an acidic environment (pH 5.5) to function, mycobacteria viability is impacted, rendering the test inaccurate. But in addition to the false resistance caused by growth restriction by acidic liquid media, the BACTEC MGIT 960 system is regarded as the primary reference approach. It requires an expensive instrument that restricts its routine application in resource-poor situations. The attempt to cure tuberculosis is hampered by the absence of PZA susceptibility testing in routine diagnosis. Therefore, the identification and initiation of successful combination regimens require a dependable in-house assay for PZA susceptibility testing.[13]

To improve the recovery of mycobacteria cultivated on solid or liquid media, a biphasic medium was created. Additionally, using liquid media can speed up *M. tuberculosis* growth; a liquid culture is less expensive than an automated liquid culture and is more susceptible to contamination than a solid culture. For PZA susceptibility testing, Gonzalo et al initially created a biphasic medium consisting of a semisolid Kirchner medium and Lowenstein-Jensen medium, which has demonstrated 95% reproducibility.[13]

The current study aimed to determine the prevalence of PZA resistance in MDRTB patients at North Karnataka, India.

METHODS

The current study was approved by institutional ethics committee (Ref No: JSS/MC/PG/0040/2022-23 Dated 05.04.2023).

At C and DST Lab KMCRI, Hubli, this prospective study was carried out over a period of January 2023 to December 2023. The study included every clinical specimen that was brought to the centre for follow-up or for newly diagnosed cases that needed programmatic treatment of DRTB. The study did not include salivary or inadequate samples or samples that met the center's SOP's rejection criteria. The investigation included a total of 11104 clinical specimens with probable MDR-TB that were received at the referral site.

A TB lab with adequate negative pressure equipment serves as the study site. For DST, smear preparation, and specimen processing, standard safety procedures were followed. For digestion, decontamination, and concentration, all specimens were treated using the conventional NALC-NaOH procedure. About 2 to 3 millilitres of phosphate buffer (pH 6.8) were used to resuspend the concentrated sediment, which was then carefully mixed. Regardless of the outcomes of the smear microscopy, a smear was prepared for acid-fast staining. The specimens were then exposed to liquid culture, and those that tested positive underwent DST using MGIT.

Direct susceptibility testing using Mgit 960

The PZA M960 assay is carried out at the referral centre in accordance with the manufacturer's instructions. The inoculum for PZA susceptibility testing was made from the leftover pellet from the MTB growth positive tube, which was suspended in phosphate buffer (pH 6.8) until it reached a final volume of 2 ml.[14] The resuspended pellet was diluted 1/10, and 0.5 ml of it was inoculated into the control tube, which also contained the PZA enrichment supplement and polymyxin B, amphotericin B, nalidixic

acid, trimethoprim, and azlocillin (PANTA). Meanwhile, 0.5 ml of the undiluted resuspended pellet was inoculated into the tube that contained 100 gm/ml PZA. As per the 21-day procedure for PZA susceptibility testing, tubes were incubated in the Bactec 960 MGIT device. The MGIT instrument's direct DST results were noted as either susceptible (S) or resistant (R).

For quality control, the *M. tuberculosis* H37Rv strain was tested using every batch of MGIT 960 PZA medium and drug package. A subculture on blood agar was used to screen for contamination in all positive MGIT tubes. Ziehl Neelson stain was used to confirm the presence of acid-fast bacilli in the smear made from positive tubes, and Gram stain was used to rule out contamination.

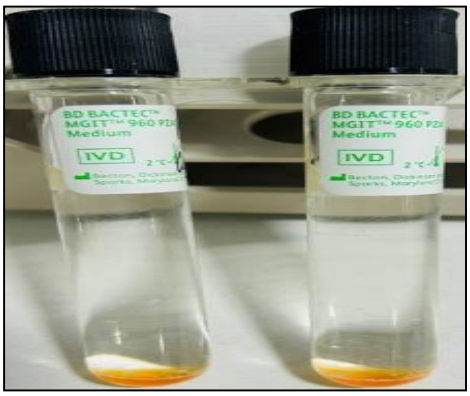

Figure 1: Representing the resistance.

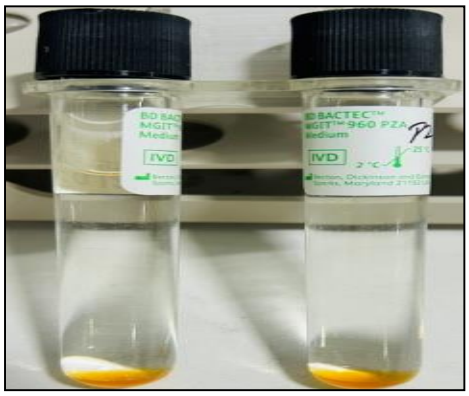

Figure 2: Representing the sensitivity.

RESULTS

11104 diagnostic samples were tested for First Line LPA at the C and DST Lab KMCRI, Hubli. January 2023 to December 2023. All samples were taken from a patient who had symptoms that pointed to active pulmonary tuberculosis. Of the 11104 samples, 207 (1.8%) were found to be MDR, 101 (0.9%) were mono rifampicin resistant and 619 (5.5%) were mono isoniazid resistant. 609 samples were then cultured using the BACTECTM MGITTM 960 method. The *M. tuberculosis* complex was rapidly detected using the Bioline™ TB Ag MPT64 test.

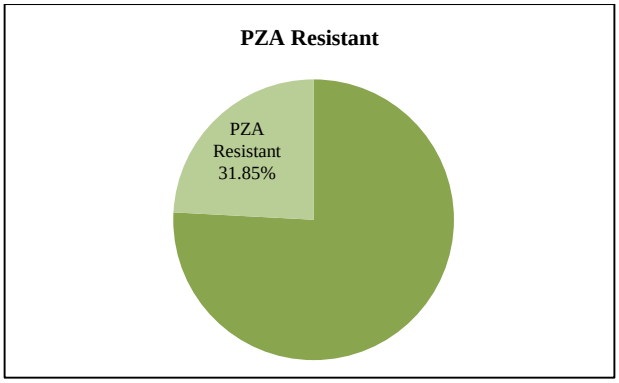

Figure 3: Graph representing PZA resistant.

MGIT-based susceptibility testing was used to determine the PZA susceptibility for 609 isolates. Of the 609 isolates, 194 (31.8%) were found to be resistant to PZA.

Of the 194 PZA-resistant patients in our research, 127 (65.4%) were cured, 25 (12.8%) died, 14 (7.2%) were lost to follow-up, and 28 (14.4%) had their regimens altered.

Table 1: Table representing the outcomes of the patients.

Outcomes	Frequency (%)
Cured	127 (65.4)
Died	25 (12.8)
Lost to follow-up	14 (7.2)
Regimens altered	28 (14.4)

DISCUSSION

Growing drug-resistant disease scenarios present a significant challenge to the global control and management of tuberculosis (TB). One of the most important first-line medications for treating tuberculosis is pyrazinamide (PZA). Relapse rates are significantly decreased, the duration of treatment is shortened from 9-12 months to 6 months, and patients infected with bacillary strains resistant to at least isoniazid and rifampicin are treated.[11]

Its discovery in 1948 during a structure-activity relationship investigation in an in vivo screen of nicotinamide derivatives is one of its distinctive and special features.[15] We now know that PZA is a prodrug that is activated by the PZase gene, which is encoded by pncA, a gene that is not essential for *M. tuberculosis*. Similar to certain other non-essential target genes, pncA mutations can frequently have a negative impact on enzymatic activity. This can be because of SNPs in essential amino acid residues that are crucial for catalysis, found at substrate or metal binding sites, or crucial for protein stability and structure. Remarkably, PZAr isolates with pncA mutations do not appear to be linked to a decline in bacterial fitness, either in vitro or in vivo. Additionally, there is minimal evidence of genetic drift, even though no

selective pressure for this gene has been seen. Because PZase is crucial to the recycling pathway of NAD rather than its production, it may be the cause of the lack of discernible impact on the organism's overall fitness.[2]

PZA's potent sterilising action results from its capacity to eliminate a population of *Mycobacterium tuberculosis* persisters that other drugs are unable to eradicate. Since administering PZA for more than two months does not seem to provide any further benefits, it is utilised during the initial two-month intensive phase of the six-month therapy. This is most likely due to the fact that inflammation that causes an acidic environment in the lesions subsides after two months. PZA is the only medication that cannot be substituted without compromising treatment effectiveness, according to more recent attempts to identify the best drug combinations with novel pharmacological possibilities for reducing the duration of TB treatment in the mouse model.[4]

The pncA gene in *M. tuberculosis* encodes the enzyme pyrazinamidase (PZase)/nicotinamidase, which transforms the prodrug PZA into the active form pyrazinoic acid (POA). The pure recombinant *M. tuberculosis* PncA is a monomer enzyme that contains Mn2+ and Fe2+. PZA conversion to POA may follow a similar process to the nitrilase superfamily, where active site cysteine nucleophilically attacks to form a tetrahedral intermediate that collapses when ammonia is lost and the thioester bond is then hydrolysed by water.[4]

PZA resistance was 31.8% in our study; this is a lower rate in relation to the worldwide burden. Compared to Zhejiang Province (43.1%), Shanghai (38.5%), Thailand (49.0%), the United States (38.0%), and Beijing (57.7%), Ningbo had a higher PZA resistance rate of 59.1%.[18,19]

PZA's crucial function in the treatment of tuberculosis emphasises the necessity of precise and prompt identification of its resistance. Since PZA is being used in the DOTS plus program to treat MDR-TB in addition to being first-line drugs, it is now more important than ever to test isolates of *M. tuberculosis* for PZA susceptibility. The length of therapy has been shortened to six months with the use of PZA in the treatment plan. It destroys semi-dormant tubercle bacilli that live in acidic environments and has strong sterilising activity. However, determining a person's susceptibility to PZA is a time-consuming process that calls for a high level of expertise. PZA is a vital first-line anti-tuberculosis drug that is essential to the therapeutic management of MDR-TB.[16,17] Considering PZA's distinct effect, identifying PZA in MDR-TB patients is crucial for initiating PZA in these refractory patients' treatment plans.

One of the study's limitations is that more research is required to better link all PZA resistance with mutation patterns, such as pncA mutations, and to increase the resolution of PZA DST determination by molecular biology.

CONCLUSION

More research is required to overcome or supplement the limits of phenotypic susceptibility assessments, increase the resolution of PZA DST determination by molecular biology, and unambiguously correlate all pncA mutations to a PZA phenotype. Given the importance of PZA in the treatment of MDR-TB, direct pncA sequencing will enable the prompt identification of PZA resistance and enable the prudent use of PZA in the management of MDR-TB that is susceptible to PZA.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee (Ref No: JSS/MC/PG/0040/2022-23 Dated 05.04.2023)

REFERENCES

1. Kushner S, Dalalian H, Sanjurjo JL, Bach Jr FL, Safir SR, Smith Jr VK, et al. Experimental chemotherapy of tuberculosis. II. The synthesis of pyrazinamides and related compounds. J Am Chem Soc. 1952;74(14):3617-21.
2. Stoffels K, Mathys V, Fauville-Dufaux M, Wintjens R, Bifani P. Systematic analysis of pyrazinamide-resistant spontaneous mutants and clinical isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2012;56(10):5186-93.
3. Zhang Y, Shi W, Zhang W, Mitchison D. Mechanisms of pyrazinamide action and resistance. Microbiol Spectr. 2014;2(4):10-128.
4. Zhang Y, Shi W, Zhang W, Mitchison D. Mechanisms of pyrazinamide action and resistance. Microbiol Spectr. 2013;2:1-12.
5. Njire M, Tan Y, Mugweru J, Wang C, Guo J, Yew W, et al. Pyrazinamide resistance in Mycobacterium tuberculosis: review and update. Adv Med Sci. 2016;61:63-71.
6. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. Int J Tuber Lung Dis. 2003;7:6-21
7. Tam KK, Leung KS, Siu GK, Chang KC, Wong SS, Ho PL, et al. Direct detection of pyrazinamide resistance in Mycobacterium tuberculosis by use of pncA PCR sequencing. J Clin Microbiol. 2019;57(8):10-128.
8. WHO. Global tuberculosis report 2020. Available from: https://www.iom.int/sites/g/files/tmzbdl486/files/documents/Global-TB-Report-2020.pdf. Accessed on 2 August 2024.
9. WHO. Global Tuberculosis Report 2019. Available from: https://www.who.int/publications/i/item/9789241565714. Accessed on 2 August 2024.
10. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, Van Soolingen D, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet. 2010;375(9728):1830-43.

11. Abdul Azeem TA, Krishna Karthik MVS KC, Sharath BN. Pyrazinamide resistance among multi drug resistant tuberculosis patients in Karnataka: cross sectional study from a referral centre. J Pulmonol Res Rep. 2023;151:2-4.
12. Shi J, Su R, Zheng D, Zhu Y, Ma X, Wang S, et al. Pyrazinamide resistance and mutation patterns among multidrug-resistant *Mycobacterium tuberculosis* from Henan Province. Infect Drug Resist. 2020;13:2929-41.
13. Thuansuwan W, Chuchottaworn C, Nakajima C, Suzuki Y, Chaichanawongsaroj N. Biphasic medium using nicotinamide for detection of pyrazinamide resistance in *Mycobacterium tuberculosis*. Antibiotics. 2024;13(6):563.
14. Becton, Dickinson and Company. BD BACTEC™ MGIT™ 960 PZA kit for the antimycobacterial susceptibility testing of *Mycobacterium tuberculosis*. Package insert L005686JAA(01) 2014-03. Becton, Dickinson and Company, Sparks, MD; 2019.
15. Kushner S, Dalalian H, Cassell RT, Sanjurjo JL, McKenzie D, Subbarow Y. Experimental chemotherapy of tuberculosis. I. Substituted nicotinamides. J Organic Chem. 1948;13(6):834-6.
16. Budzik JM, Jarlsberg LG, Higashi J, Grinsdale J, Hopewell PC, Kato-Maeda M, Nahid P. Pyrazinamide resistance, Mycobacterium tuberculosis lineage and treatment outcomes in San Francisco, California. PLoS One. 2014;9(4):e95645.
17. Miotto P, Tessema B, Tagliani E, Chindelevitch L, Starks AM, Emerson C, et al. A standardised method for interpreting the association between mutations and phenotypic drug resistance in Mycobacterium tuberculosis. Eur Respir J. 2017;50(6).
18. Xia Q, Zhao LL, Li F, Fan YM, Chen YY, Wu BB, et al. Phenotypic and genotypic characterization of pyrazinamide resistance among multidrugresistant mycobacterium tuberculosis isolates in Zhejiang, China. Antimicro Agents Chemother 2015;59:1690-5.
19. Gu Y, Yu X, Jiang G, Wang X, Ma Y, Li Y, et al. Pyrazinamide resistance among multidrug-resistant tuberculosis clinical isolates in a national referral center of China and its correlations with pncA, rpsA, and panD gene mutations. Diagn Microbiol Infect Dis. 2016;84:207-11.

Cite this article as: Dhapalapur NR, Nirmala, Hegde AM. Detection of pyrazinamide resistance among multidrug resistant tuberculosis in North Karnataka. Int J Res Med Sci 2025;13:1499-503.