Volume 71, No. S1 | 2024

ISSN: 0019-5707

Indian Journal of Tuberculosis

Volume 71 Number S1 2024

Table of Contents

Contents Continued on IBC

Volume 71, No. S1 | 2024

Indian Journal of TUBERCULOSIS

ELSEVIER

Indian Journal of TUBERCULOSIS

The Tuberculosis Association of India

Multi-drug Resistance in Tuberculosis

Guest Editors:
Vijay Kumar Arora
Kamal Kishore Chopra

Contents Continued from OBC

Contents Continued on BM

INDIAN JOURNAL OF TUBERCULOSIS

EDITORIAL BOARD

Indian Journal of Tuberculosis

Volume 71 Number S1 2024

Table of Contents

Indian Journal of Tuberculosis 71 (2024) S1–S2

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

MDR TB: New drugs, research and policy

To manage a case of MDR TB, we not only require quick and accurate diagnosis but very effective and short duration treatment. Nowadays, we have got several sensitive and WHO endorsed tests for confirming case of MDR TB. But for treatment purpose, there have been several recommendations after the discovery of newer drugs, in composition and duration of treatment regimens. Here comes the role of researchers and policy makers to include new evidence base recommendations in national programs at the earliest to give benefit to MDR patients.

Drug-resistant tuberculosis (DR-TB) continues to pose a significant global health challenge, with approximately 410,000 MDR/RR-TB (MDR: A TB patient whose biological specimen is resistant to both H and R with or without resistance to other first-line anti-TB drugs. RR: A TB patient, whose biological specimen is resistant to R, detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs.) Cases reported in 2021 (95% UI: 370,000–450,000).[1] Although highly sensitive, rapid molecular tests are available, it's availability and sensitization among treating physicians is still lacking specially in private sector, so its desired advantage is still not achieved. India alone accounted for 26% of the total global cases of MDR/RR-TB in 2022. The treatment success rate for drug-resistant TB in 2022 was 63% globally, reflecting an increase from the 60% recorded in 2019 and a notable improvement from the 50% reported in 2012(1). Before making policies for drug resistant TB management, sincere efforts should be made for prevention of emergence of drug resistance. For this, there should be strong policies for drug adherence, using newer rapid diagnostics and newer drug combinations for management of drug sensitive cases, so that there are less chances of emergence of drug resistance. Studies are going on for formulating a universal (both for drug sensitive and drug resistant cases) regimen of shorter (4-month duration) which should be fast tracked.

Treating MDR/RR-TB has proven to be difficult in the past few decades due to several factors, including the extended duration of treatment, which can span up to 20–24 months, along with concerns regarding toxicity, financial burdens, and suboptimal treatment outcomes. After the availability of two new drugs (bedaquiline and delamanid), the World Health Organization in March 2019 endorsed the possibility of treating MDR-TB patients with a full oral regimen lasting 9–10 months.[2]

The current guidelines for the programmatic management of DR-TB in India were released on March 24, 2021 and are in line with the WHO Consolidated Guidelines on Tuberculosis (TB), Module 4: Treatment 2020.[3,4] The guidelines recommend the use of the following two regimens for the treatment of MDR/RR-TB. The shorter regimen (shorter all-oral bedaquiline-containing regimen) 4–6 (bedaquiline [BDQ] (6 m)-levofloxacin [Lfx]/moxifloxacin [MFX]-clofazimine [Cfz]-pyrazinamide [Z]-Ethambutol [E]-isoniazid high dose [Hh]-Ethionamide [ETo]/5 Lfx/Mfx-Cfz-Z-E and longer regimen: 18 Bdq(6 m)-Lfx/Mfx-Lzd-Cfz-Cs (18-month treatment combination composed of Bdq for the first intensive phase 6 months and levofloxacin or moxifloxacin, Lzd, Cs and Cfz for next 18 months) if patient is not eligible for shorter regimen. This regimen has shown promising results in trial conditions, up to 80% success rate, but when used in programmatic conditions, we are getting only 60 to 65% success rate. Signifying there are other factors also playing their role, like adherence to treatment, quality and availability of drugs, training status of treating physicians and timely and clear guidelines from policymakers. Another factor observed is rising level of fluoroquinolones resistance in many states, 35 to 45% in Delhi, making patient not eligible for shorter regimen.

The new recommendations have been implemented with the hope that the increased availability of these newer therapeutic drugs will lead to notable improvements in treatment outcomes. However, the exclusion from short-course DR regimens, persistent complexities, and toxicities associated with existing treatment protocols pose challenges to treatment adherence, alongside the looming risk of resistance emergence to newer drugs. These factors emphasize the need for continuous efforts in drug and regimen development against DR-TB.

Several ongoing clinical studies aim to further evaluate the efficacy and safety of these new and repurposed drugs in regimens of shortened duration. Notable among these are: 1) the 6-month regimen based on bedaquiline, pretomanid, and linezolid (BPaL) in combination with moxifloxacin (BPaLM), evaluated in the TB-PRACTECAL randomized clinical trial; 2) the 6-month regimens based on the BPaL combination with decreased exposure to linezolid (lower dosing or shorter duration) evaluated in the ZeNix study, and 3) the modified all-oral shorter regimens (6–9 months or 9–12 months) containing all three Group A drugs, evaluated in the NeXT trial.[5–8]

Based on the evidence generated by the trials, in December 2022, the WHO released the updated WHO Consolidated Guidelines on Tuberculosis treatment.[9] These updated Guidelines in addition to the previous shorter (9 month), all-oral, BDQ containing regimen and longer regimen (18 months), also recommend the use of the 6-month BPaLM regimen (bedaquiline, pretomanid, linezolid 600 mg, and moxifloxacin) in place of the previous shorter or longer regimens for MDR/RR-TB. This regimen can be used as BPaL, i.e., In pre-XDR-TB patients with

https://doi.org/10.1016/j.ijtb.2024.06.005
Received 12 May 2024; Accepted 6 June 2024
Available online 13 June 2024

documented resistance to fluoroquinolones (FQs). Although BPaL regimen has shown 89% success rate and is being used in many countries with good results, still it is not being used in NTEP in India. Here comes the role of researchers to document its success in Indian patients and policy makers to take prompt decisions on such important issues.

Despite the promising results, the uptake of these new regimens, until recently, has been slow and problematic in many countries, including India. There are reasons for less uptake as factors that include logistical, programmatic, and regulatory approvals. Pre-approval pharmaceutical research and development (R&D) trials are primarily driven by the private sector with a focus on quick market approval. This often leads to gaps in evidence for guiding clinical practice. However, it is important to recognize that early regulatory approvals shift the responsibility of generating necessary safety and efficacy data from market authorization holders to healthcare organizations. Similarly, there are many new drugs in pipeline for treatment of Drug Resistant TB. Efforts should be made to conduct trials with combination of drugs with newer drugs (in last stage of development), so that these may be quickly introduced in the Programme. Regarding TPT for contacts of DR TB case, although NTEP has recommended Levofloxacin, but resistance to fluoroquinolones is reported to vary from 25 to 40% in different states. So, different combinations with newer drugs should be tried as prevention with proper drugs is more important than treating MDR cases. Similarly, efforts on development of an effective vaccine against the disease should be fast tracked so as to prevent it.

In conclusion, managing MDR TB requires several key factors. Firstly, there should be access to reliable, rapid, and widely available drug sensitivity testing. Following diagnosis, a proper referral mechanism to specialized centers is essential for expert management. Pretreatment evaluation facilities are also crucial. National TB program guidelines play a vital role in determining suitable regimens, as they are evidence-based and regularly updated to incorporate the latest findings. These guidelines are often based on recommendations from researchers derived from high-quality clinical trials. Lastly, the discovery and availability of new drugs to shorten treatment duration is important. By effectively addressing these aspects, we can more effectively manage MDR cases.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. *World Health Organization Global Tuberculosis Report 2023*. Geneva: World Health Organization; 2023. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023.
2. World Health Organization. *WHO Consolidated Guidelines on Drug-Resistant Tuberculosis Treatment*. Geneva: World Health Organization; 2019. https://www.who.int/publications/i/item/9789240063129.
3. *Guidelines for the Programmatic Management of Drug Resistant Tuberculosis in India*. Centeral TB Division Ministry of Health and Family Welfare, GoI; 2021. https://tbcindia.gov.in/showfile.php?lid=3590.
4. World Health Organization WHO consolidated guidelines on tuberculosis. *Module 4: Treatment - Drug-Resistant Tuberculosis Treatment*. Geneva: World HealthOrganization; 2020. https://www.who.int/publications/i/item/9789240007048.
5. Berry C, du Cros P, Fielding K, et al. TB-PRACTECAL: study protocol for a randomised, controlled, open-label, phase II–III trial to evaluate the safety and efficacy of regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug-resistant tuberculosis. *Trials*. 2022;23:484. https://doi.org/10.1186/s13063-022-06331-8.
6. Nyang'wa BT, Berry C, Kazounis E, et al. A 24-week, all-oral regimen for rifampin-resistant tuberculosis. *N Engl J Med*. 2022;387:2331–2343. https://doi.org/10.1056/NEJMoa2117166.
7. Esmail A, Oelofse S, Lombard C, et al. An all-oral 6-month regimen for multidrug-resistant tuberculosis: a multicenter, randomized controlled clinical trial (the NExT Study). *Am J Respir Crit Care Med*. 2022;205:1214–1227. https://doi.org/10.1164/rccm.202107-1779OC.
8. Conradie F, Bagdasaryan TR, Borisov S, et al. Bedaquiline–pretomanid–linezolid regimens for drug-resistant tuberculosis. *N Engl J Med*. 2022;387:810–823. https://doi.org/10.1056/NEJMoa2119430.
9. *World Health Organization WHO Consolidated Guidelines on Tuberculosis. Module 4: Treatment - Drug-Resistant Tuberculosis Treatment, 2022 Update*. Geneva: World Health Organization; 2022. https://www.who.int/publications/i/item/9789240063129.

V.K. Arora
TB Association of India, 3 Red Cross Rd, Gokul Nagar, Sansad Marg Area, New Delhi, Delhi, 110001, India
E-mail address: vijaykumar1945@gmail.com.

Kamal Kishore Chopra*, Sanjay Rajpal, Zeeshan Sidiq
New Delhi Tuberculosis Centre, Jawaharlal Nehru Marg, Delhi Gate, New Delhi, 110002, India

* Corresponding author.
E-mail addresses: chopra_drkk@yahoo.co.in (K.K. Chopra), sanjayrajpalin@yahoo.com (S. Rajpal), zeeshansidiq@gmail.com (Z. Sidiq).

Indian Journal of Tuberculosis 71 (2024) S3–S4

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Is it the time for abandoning longer regimens for drug-resistant tuberculosis

ARTICLE INFO

Keywords
Drug resistant
Tuberculosis
Treatment
Shorter regimen

Funding/grant

Nil.

Tuberculosis (TB) continues to be a major public health problem, causing significant morbidity and deaths globally. In 2022, an estimated 10.6 million individuals suffered from tuberculosis (TB) worldwide.[1] Out of this an estimated 410 000 people developed multidrug-resistant or rifampicin resistant TB (MDR/RR-TB). In the same year, about 30% of the world's drug-resistant tuberculosis (DR-TB) cases were recorded in India.[1] As per the targets fixed by the End TB Strategy of World Health Organisation (WHO) for eradicating TB, there should be 80% and 90% reductions in incidence rates, as well as 90% and 95% reductions in fatality rates, by 2030 and 2035, respectively.[1] The Indian government's goal of eliminating tuberculosis by 2025 is a commendable undertaking.

The management of DR-TB has seen better outcomes over the years due to introduction of newer medications like bedaquiline (Bdq) and delamanid (Dlm), as well as use of repurposed drugs like linezolid (Lzd) and clofazimine (Cfz), which form the main components of DR-TB regimens. WHO is focusing its efforts on increasing treatment success rates. In early May 2022, the World Health Organisation issued a rapid communication mentioning various shorter oral regimens for MDR-TB that had demonstrated good success internationally.

Previously, resistance to rifampicin and isoniazid forced the use of longer courses of second-line medicines, which have been linked to poor outcomes, greater medication costs, and toxicity. For many years, fluoroquinolones (FQs) and injectable medicines like kanamycin, capreomycin, and amikacin have been the main components of MDR-TB treatment. MDR-TB was assumed to require a lengthier treatment schedule due to a lack of additional medications with the high bactericidal activity comparable to that of isoniazid (INH) and sterilising action of rifampicin for drug sensitive tuberculosis (DS-TB).

Under the national programme in India, the first patient was initiated on MDR-TB regimen in August 2007. Initially a standard treatment regimen for MDR-TB consisted of 6–9 months of intensive phase with six drugs followed by 18 months of continuation phase with four drugs. A standard treatment regimen for extensively drug resistance (XDR-TB) consisted of 6–12 months of intensive phase with seven drugs followed by 18 months of continuation phase with six drugs was being offered under the national programme. These regimens being far more complex and longer in duration were associated with poor treatment success rates and high likelihood of lost to follow up due to more adverse drug reactions. In India, just 48% of patients received a successful therapeutic outcome. The outcomes were not good due to longer duration of treatment, use of injectable drugs with greater side effects resulting in poor adherence to treatment.

Based on 2016 WHO guidelines, India adopted shorter regimen for DR-TB in 2017 for those patients who had not previously taken second line drugs for more than one month and with documented sensitivity to FQs and second line injectables (SLI). The Bangladesh study and the STREAM (Standard Treatment Regimen of *Anti*-TB Drugs for Patients with MDR TB) Stage 1 trial formed the basis of this regimen.[2,3] STREAM was a phase 3 randomised control trial (RCT) which showed that shorter regimen was not inferior to lengthier regimen in terms of outcomes (78.8% versus 79.8%).[2,3] However, there were some disadvantages to the shorter regimen. Drugs like aminoglycosides, high dose INH, and ethionamide which need to be given for 4–6 months and having poor safety profile are a part of this regimen. Logistical challenges in the form of multiple intramuscular drug injections lead to poor adherence. The data on the efficacy of shorter MDR/RR-TB regimens in certain scenarios, including different drug sensitivity patterns, human immunodeficiency virus status, extrapulmonary involvement (excluding lymph nodes and pleura), severe forms of TB, and pregnancy is non-existent which adds to the worry.

There is also evidence that the outcome is poor in patients started on shorter regimen who have documented resistance to fluoroquinolones and pyrazinamide either in the beginning or later on during ongoing treatment.[4] Only 25%–33% of MDR/RR-TB patients satisfy the eligible clinical and microbiological criteria for shorter regimen as per data from most countries.[4] In 2019 WHO consolidated guidelines of DR-TB mentioned more simplified regimens including all oral longer regimen. The patients excluded from shorter regimen were to be started on all oral longer regimen.

Certain eligible patients diagnosed with MDR-TB or RR-TB who have not taken second-line medicines previously for more than one month

https://doi.org/10.1016/j.ijtb.2024.06.008
Received 23 January 2024; Received in revised form 18 June 2024; Accepted 20 June 2024
Available online 21 June 2024

and in whom there is no FQ resistance are eligible for a shorter all oral bedaquiline-containing regimen of 9–12 months duration as per recommendation by WHO given in 2020. The data of patients enrolled under the national programme in South Africa formed the basis of this shorter oral bedaquiline-containing regimen. Injectable agent was dropped and substituted with bedaquiline as compared to injectable containing shorter regimen. It revealed favourable results, with Bdq-containing shorter oral regimen reporting 73% treatment success as compared to 60% with injection containing shorter regimen.[5]

Further, NiX study was done in patients with extensively drug-resistant tuberculosis and patients with multidrug-resistant tuberculosis that was not responsive to treatment or for which a second-line regimen had been discontinued because of side effects. Bedaquiline and Pretomanid started in standard dosages for 26 weeks and linezolid at a dose of 1200 mg daily for up to 26 weeks in this trial. At 6 months following the conclusion of treatment, 10% of patients had an unfavourable result due to treatment failure (bacteriologic or clinical) or recurrence during follow-up, whereas 90% had a positive outcome. The research also showed the harmful effects of linezolid on peripheral neuropathy (81% of patients) and myelosuppression (48%).[6]

In view of higher adverse drug reactions in Nix trial with linezolid, ZeNix trial evaluated different dosages and duration of linezolid among similar patients as in Nix trial. Among participants who received bedaquiline–pretomanid–linezolid with linezolid at a dose of 1200 mg for 26 weeks or 9 weeks, or 600 mg for 26 weeks or 9 weeks, 93%, 89%, 91%, and 84%, respectively, had a favourable outcome. Peripheral neuropathy occurred in 38%, 24%, 24%, and 13%, respectively; myelosuppression occurred in 22%, 15%, 2%, and 7%, respectively. Optic neuropathy developed in 4 participants (9%) who had received linezolid at a dose of 1200 mg for 26 weeks. Overall regimen containing 600 mg linezolid had most acceptable results.[7]

Another trial, TB-PRACTECAL evaluated the efficacy and safety of three 24-week, all-oral regimens for the treatment of RR-TB. In the BPaL regimen, linezolid was given at a dose of 600 mg daily for 16 weeks and the dosage was halved for next 8 weeks along with bedaquiline and pretomanid in standard dosages. The BPaLM regimen included BPaL plus moxifloxacin at a dose of 400 mg daily for 24 weeks, and the BPaLC regimen included BPaL plus clofazimine at a dose of 100 mg daily (or 50 mg if the patient weighed $<$30 kg) for 24 weeks. A successful treatment outcome was achieved in 89% of participants, at the end of treatment, in the BPaLM arm of the TB-PRACTECAL.[8]

Based on above trial results, WHO rapid communication in early May 2022 mentioned various shorter oral regimens for MDR-TB which have shown good results globally. These regimens include a new 6-month regimen based on bedaquiline, pretomanid and linezolid (BPaL) in combination with moxifloxacin (BPaLM). The basis of this regimen is the results of TB-PRACTECAL trial. WHO also recommended BPaL to be used when there is documented resistance to flouroquinolones. BPaL regimen has shown good results in different dosages and durations of linezolid in many trials including the NiX and ZeNix trial.[6,7]

In BEAT-TB trial – two newer drugs bedaquiline, and delamanid along with two repurposed drugs linezolid, and clofazimine was given for 24–36 weeks. The eligible patients were adults with pulmonary MDR-TB with fluoroquinolone resistance or/and aminoglycoside resistance. In outcome 91% patients had favourable outcome at treatment end defined as two consecutive negative cultures taken four weeks apart. Myelosuppression and peripheral neuropathy was seen in 52% and 42% of patients, respectively, and QTc by Fredericia prolongation of $>$500msec was seen in none.[9]

Furthermore, a few trials are underway to develop shorter regimens capable of treating both DS-TB and DR-TB diseases while preferring a universal treatment strategy. These comprehensive shorter regimens are also termed as Pan-TB regimens. These Pan-TB regimens consist of pretomanid as a key drug.

To conclude, it is high time that we accelerate our efforts so that the menace of TB, especially DR-TB, can be tackled at a rate that our country needs. The gradual introduction of modified, shorter DR-TB regimens at programmatic level needs to be considered. Perhaps time has come to have shorter regimen for DR-TB as standard of care and to say goodbye to longer regimen except in situations where shorter DR-TB regimen cannot be given or are contraindicated.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. *Global Tuberculosis Report*; 2023 [Internet]. [cited 2023 Nov 25]. Available from: https://www.who.int/publications-detail-redirect/9789240083851.
2. Aung KJM, Van Deun A, Declercq E, et al. Successful "9-month Bangladesh regimen" for multidrug-resistant tuberculosis among over 500 consecutive patients. *Int J Tubercul Lung Dis*. 2014 Oct;18(10):1180–1187.
3. Nunn AJ, Phillips PPJ, Meredith SK, et al. A trial of a shorter regimen for rifampin-resistant tuberculosis. *N Engl J Med*. 2019 Mar 28;380(13):1201–1213.
4. Abidi S, Achar J, Assao Neino MM, et al. Standardised shorter regimens *versus* individualised longer regimens for rifampin- or multidrug-resistant tuberculosis. *Eur Respir J*. 2020 Mar;55(3), 1901467.
5. Ndjeka N, Campbell JR, Meintjes G, et al. Treatment outcomes 24 months after initiating short, all-oral bedaquiline-containing or injectable-containing rifampicin-resistant tuberculosis treatment regimens in South Africa: a retrospective cohort study. *Lancet Infect Dis*. 2022 Jul;22(7):1042–1051.
6. Conradie F, Diacon AH, Ngubane N, et al. Treatment of highly drug-resistant pulmonary tuberculosis. *N Engl J Med*. 2020 Mar 5;382(10):893–902.
7. Conradie F, Bagdasaryan TR, Borisov S, et al. Bedaquiline–pretomanid–linezolid regimens for drug-resistant tuberculosis. *N Engl J Med*. 2022 Sep 1;387(9):810–823.
8. Nyang'wa Bt, Berry C, Kazounis E, et al. A 24-week, all-oral regimen for rifampin-resistant tuberculosis. *N Engl J Med*. 2022 Dec 22;387(25):2331–2343.
9. Padmapriyadarsini C, Vohra V, Bhatnagar A, et al. Bedaquiline, delamanid, linezolid and clofazimine for treatment of pre-extensively drug-resistant tuberculosis. *Clin Infect Dis*. 2022 Jun 29;76(3):e938–e946.

Rupak Singla*
Department of Tuberculosis and Chest Diseases, National Institute of Tuberculosis and Respiratory Diseases, New Delhi, 110030, India

Amitesh Gupta
Department of Pulmonary Medicine, Maulana Azad Medical College and Associated Lok Nayak Hospital, New Delhi, 110002, India

* Corresponding author. Department of Tuberculosis and Chest Diseases, National Institute of Tuberculosis and Respiratory Diseases, New Delhi, 110030, India.
E-mail address: drrupaksingla@yahoo.com (R. Singla).

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Case report

Linezolid induced toxic optic neuropathy in drug resistant tuberculosis-A case series

Siddharth Vallabhaneni [a], *Teena Mariet Mendonca* [b,*], *Rajesh R. Nayak* [b], *Gurudutt Kamath* [b], *Ajay Kamath* [b]

[a] *Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India*
[b] *Department of Ophthalmology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India*

ARTICLE INFO

Article history:
Received 14 November 2022
Received in revised form
15 June 2023
Accepted 22 June 2023
Available online 27 June 2023

Keywords:
Drug toxicity
Linezolid
Optic neuropathy
Tuberculosis

ABSTRACT

In developing countries like India, Linezolid is widely used for the treatment of Multi drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB). Long-term administration of Linezolid is reported to cause toxic optic neuropathy causing bilateral, progressive visual loss in patients. We report case details of three patients on anti-tubercular therapy presented to us with sudden, progressive, painless blurring of vision of both eyes the cause of which was confirmed to be toxic optic neuropathy due to linezolid. Subsequently, cessation of the drug resulted in complete visual recovery in two patients whereas one patient had minimal visual improvement due to secondary optic atrophy. Clinicians and health care workers need to be aware of sight threatening complications of Linezolid.

1. Introduction

Linezolid is used extensively for Multi drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) by the clinicians all over the world. In developing counties like India, with increasing incidence of drug-resistant Tuberculosis, there has been an increase in the usage of drugs like Linezolid to treat the disease.[1,2] However, safety profile of linezolid for long-term administration is still unclear. Toxic optic neuropathy is a disorder characterised by damage to the optic nerve due to toxins, including drugs resulting in vision impairment.[3] It is characterised by a bilateral, usually symmetrical loss of vision and abnormal colour vision.[4] Linezolid is a member of synthetic antimicrobials known as Oxalidinones, which has been reported to cause toxic optic neuropathy. We report case details of three patients on anti-tubercular therapy presented to us with sudden, progressive, painless blurring of vision of both eyes. Fundus examination, Visual evoked potentials, and Optical Coherence

* *Corresponding author*. Tel.: +91 9461495479.
E-mail addresses: siddv82@gmail.com (S. Vallabhaneni), tmendonca87@gmail.com (T.M. Mendonca), eyenayak@yahoo.com (R.R. Nayak), mgkamat@rediffmail.com (G. Kamath), ajayrkamath@yahoo.com (A. Kamath).
https://doi.org/10.1016/j.ijtb.2023.06.016

Tomography showed features of optic nerve damage, suggesting a diagnosis of toxic optic neuropathy.

2. Case report

2.1. *Patient 1*

A 40-year-old man presented to us with complaints of sudden, painless, progressive diminution of vision in both eyes for 25 days. He was a known case of Multidrug-Resistant Pulmonary Tuberculosis, diagnosed through sputum CBNAAT, and on medication for the past seven months. He had received 600 mg of Linezolid, 100 mg of Clofazimine, 100 mg of Levofloxacin, 750 mg of Cycloserine and 100 mg of Pyridoxine once daily for the past seven months. On examination, his best corrected visual acuity was Counting fingers up to 2 m in both eyes and colour vision was abnormal. Fundus examination showed mild temporal optic disc pallor in both eyes. Visual field analysis showed generalized constriction of the peripheral visual field in both eyes (Fig. 1). Visual Evoked Potential (VEP) was done, which showed prolonged P100 latencies and reduced amplitude in both eyes (Fig. 2). An Optical Coherence Tomography (OCT) was done, which revealed Retinal Nerve Fibre Layer (RNFL) thinning in the right eye, more in the inferior and temporal quadrants (Fig. 3). Linezolid-induced toxic optic neuropathy was suspected, and Linezolid was stopped after a discussion with the treating physician and was started on oral vitamin B supplements. Tab.Delamanid 200 mg per day was started instead of Tab Linezolid. The patient was followed up after four weeks after discontinuing Linezolid. Visual acuity improved to 6/24 in both eyes, and colour vision was restored.

2.2. *Patient 2*

A 26-year-old man presented with complaints of sudden, painless blurring of vision in both his eyes for the past five days. He was a known case of Pulmonary TB for which he had completed treatment one year ago. He developed fever and cough with expectoration seven months ago, when he was diagnosed to have Rifampicin resistant pulmonary TB and was started on 600 mg of Linezolid, 100 mg of Clofazimine, 100 mg of Levofloxacin, 750 mg of Cycloserine and 100 mg of Pyridoxine orally once daily. On ocular examination, his best corrected visual acuity was found to be 6/18 in both eyes. His colour vision was abnormal in both eyes. The pupils were briskly reacting to light, and the anterior segment was unremarkable. On fundus examination, the patient was found to have bilateral hyperemic optic discs with peripapillary oedema. Few superficial flame haemorrhages were noted in the peripapillary region (Fig. 4).Humphrey's visual field examination was done, which showed a centrocaecal scotoma in both eyes. Linezolid was replaced with tab Delamanid 200 mg per day, and he was started on oral vitamin B supplements. The patient was reviewed after four weeks. On examination, he had normal colour vision, and his vision had improved to 6/6 in both eyes. Fundus examination revealed resolved optic disc oedema bilaterally with no peripapillary haemorrhages.

2.3. *Patient 3*

A 32-year-old man presented with complaints of painless, progressive blurring vision in both his eyes for the past month. He was a known case of extrapulmonary TB who had initially received Isoniazid 300 mg, Rifampicin 600 mg, and

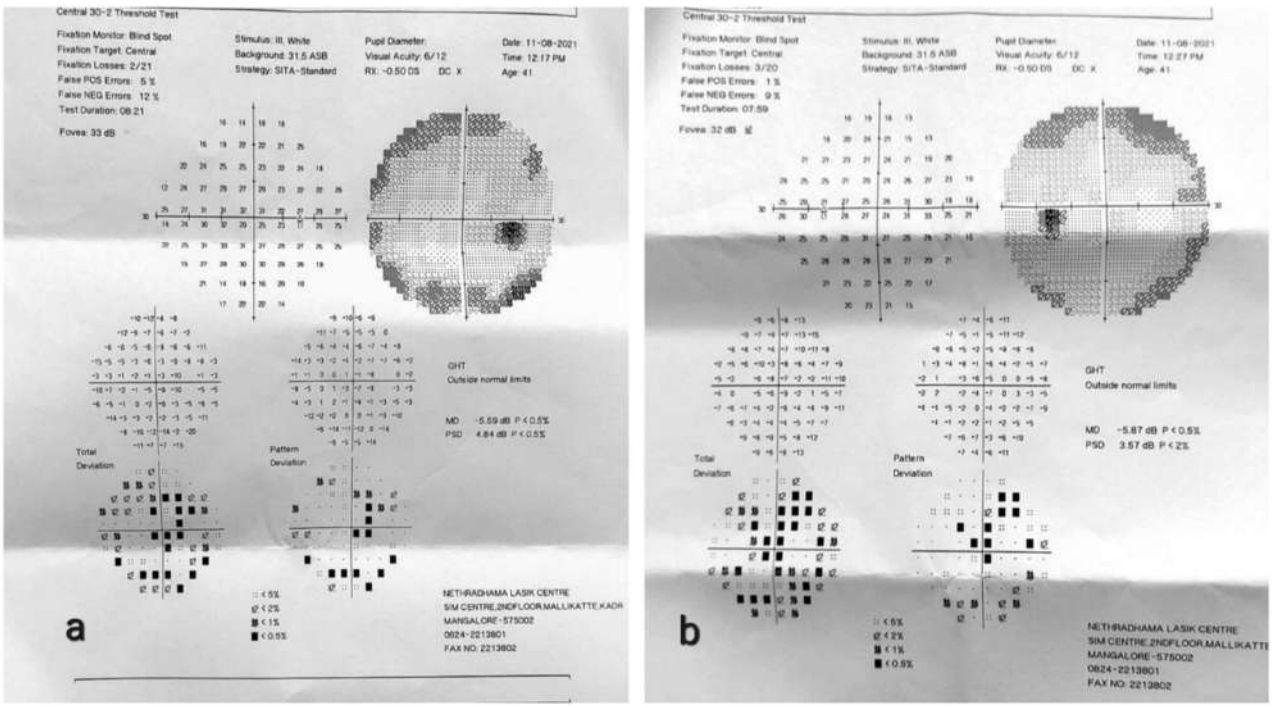


Fig. 1 – Figure showing visual fields of the right eye (a) and left eye (b) of patient 1, showing generalized visual field constriction.

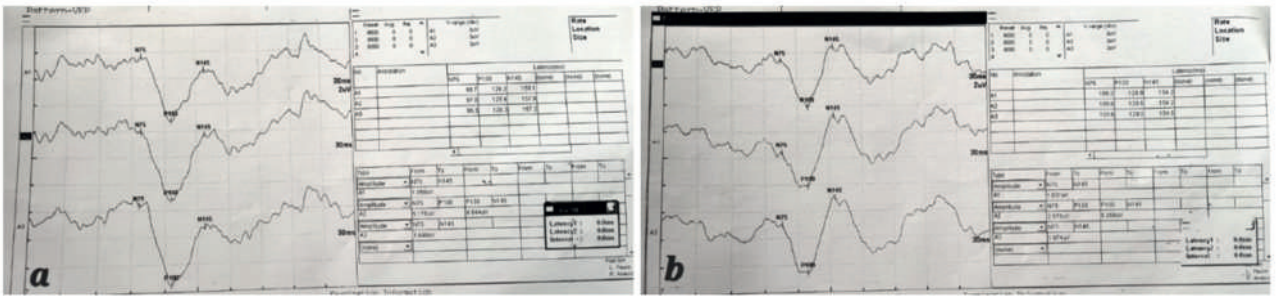


Fig. 2 – Figure showing the Visual Evoked Potentials of the right eye (a) and left eye (b) of patient 1. There is decreased amplitude and prolonged P100 latency in both eyes.

Ethambutol 1100 mg orally once daily for six months. The patient then reported blurring of vision in both his eyes. Ethambutol-induced toxic optic neuropathy was suspected, and the drug was discontinued. The patient subsequently developed pleural effusion, an examination of which revealed Rifampicin resistance. He was then started on a new ATT regimen of oral Linezolid 600 mg, Levofloxacin 100 mg, Clofazimine 100 mg, Cycloserine 750 mg, Pyridoxine 100 mg daily and Bedaquiline 200 mg 3 times per week. One month after the initiation of this new regimen, the patient developed a rapidly progressing diminution of vision and was referred to us. On examination, his visual acuity was found to be counting fingers at 1 m in both eyes. Pupils and anterior segments were unremarkable. Fundus examination revealed bilateral hyperaemic optic discs. Visual evoked potential showed prolonged P100 latencies and decreased amplitudes in both eyes (Fig. 5). Oral Linezolid was stopped, and the patient was started on oral Delamanid 200 mg per day and vitamin B supplements. On review after one month, his vision was counting fingers at 2 m in both eyes and fundus examination revealed bilateral partial optic atrophy. At 6 months follow up, patients' visual acuity recovered to 6/9 in both eyes. However, colour vision remained abnormal and fundus examination revealed partial optic atrophy.

3. Discussion

Toxic optic neuropathy is a condition, which is usually underdiagnosed, and even when diagnosed, it is at a late stage of the disease where visual recovery is not possible.

Causes of toxic optic neuropathy include tobacco and alcohol consumption, including methanol, antibiotics like chloramphenicol, sulfonamides, Linezolid, anti-tubercular drugs like isoniazid, ethambutol, streptomycin, antimalarials like chloroquine, quinine, antiarrhythmic drugs like

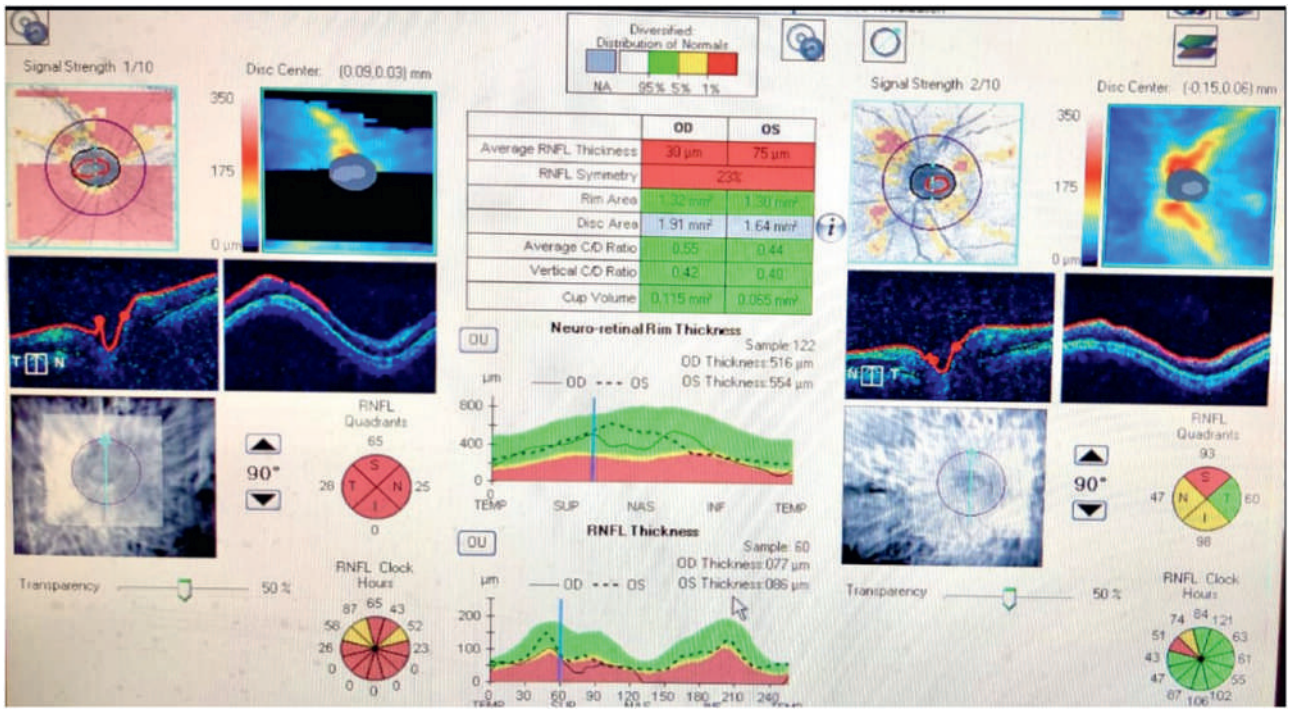


Fig. 3 – Figure showing Retinal nerve fibre layer (RNFL) OCT of both the eyes of patient 1. It shows RNFL thinning in the right eye.

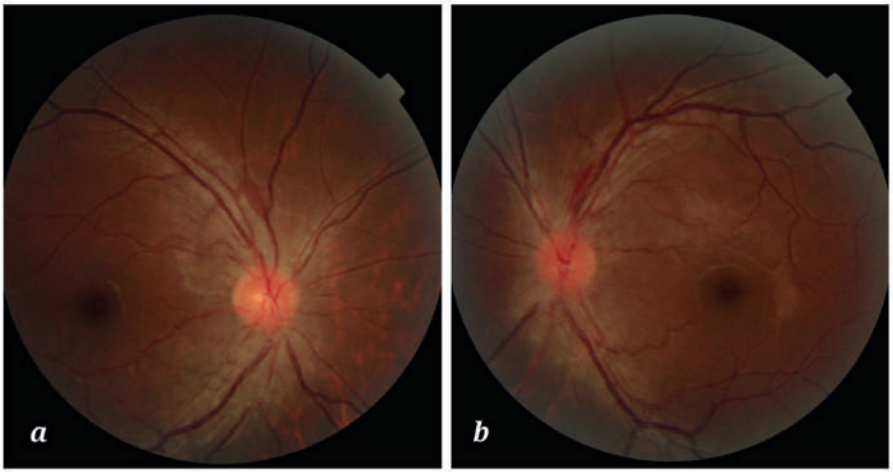


Fig. 4 – a and b shows the fundus photographs of the right and left eyes respectively of patient 2. Bilateral hyperemic optic discs with oedema and peripapillary hemorrhages are seen.

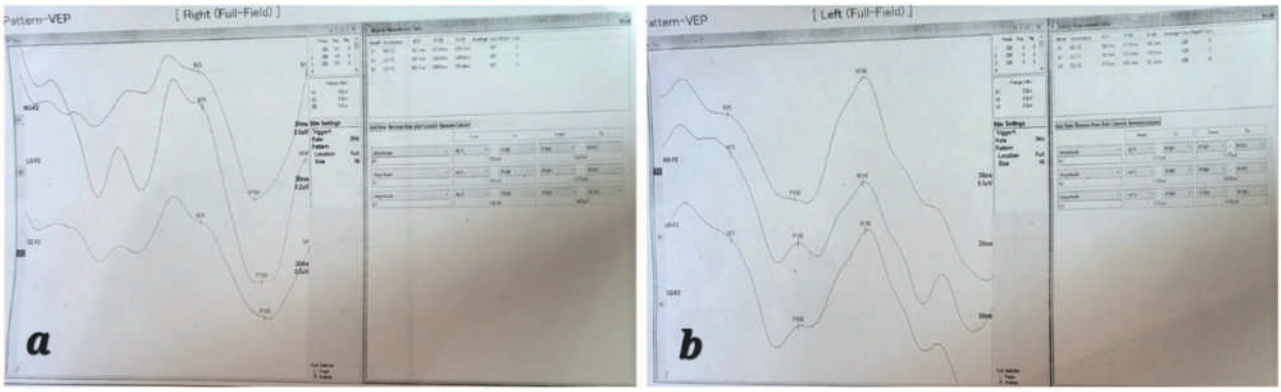


Fig. 5 – Visual evoked potential of patient 3 showing prolonged P100 latencies and decreased amplitudes of the right and left eye respectively.

amiodarone and digitalis, anticancer drugs like vincristine and methotrexate as well as heavy metals like mercury, thallium and lead.[1] Linezolid is a synthetic antimicrobial belonging to the Oxalidinone group. It was approved for the treatment of Gram-positive infections in 2000. It has been recommended by the WHO for the treatment of drug-resistant tuberculosis as a drug with unclear efficacy.[5] The mechanism of action of Linezolid is through inhibition of bacterial protein synthesis. This is achieved through specific binding of the 50s subunit of ribosomes. Although this inhibition has no effect on mammal proteins, prolonged duration of treatment can cause dysfunction by interfering with mitochondrial protein synthesis. This altered mitochondrial oxidative metabolism has been proposed to be the aetiology of optic neuropathy by Linezolid.[6] The duration of Linezolid intake may play a role in the occurrence of toxic optic neuropathy. FDA approval was given for Linezolid based on studies where the drug was administered for 28 days.[7] In previous reports, the duration of linezolid intake ranged from five to ten months, at a dose of 600 mg, either once or twice per day.[2,8] In a retrospective study by Mehta et al., it was found that out of eighty six patients with drug resistant TB, who had received Linezolid, five patients had optic neuropathy. All five patients had received 600 mg of Linezolid daily for an average of ten months and had symptoms of blurring of vision.[9] In a systematic review and meta analysis by Sotgiu et al. on the safety, efficacy and tolerability of Linezolid containing regimens in the treatment of MDR-TB, it was found that approximately one out of every two patients (58.9%) had adverse effects attributed to Linezolid. The most common adverse effects were anaemia and peripheral neuropathy, with optic neuritis occurring in 13.2% of cases.[10] In a systematic review of optic neuropathy associated with Linezolid by Brandariz-Núñez et al., thirty-three cases from twenty-six independent articles were analysed. Diminution of vision was observed in thirty of the thirty-three patients. Twenty-eight of them had bilateral diminution of vision. Optic disc changes were noted in twenty three of the twenty-nine documented cases. Once Linezolid was withdrawn, improvement in visual acuity was seen in 31 of the 33 patients.[6] In our case series, two patients had received 600 mg of Linezolid daily for more than six months, and one patient received it for one month when visual symptoms started to develop. Fundus changes were noted in all three patients. Withdrawal of Linezolid led to an improvement in visual

acuity in two patients, whereas in one patient, only a minimal improvement was observed. With the increasing incidence of MDR and XDR TB in India, physicians and ophthalmologists must be aware of the importance of monitoring patients receiving Linezolid. A baseline examination must be conducted prior to starting Linezolid with monthly follow-up. The patients must be educated regarding the possibility of vision loss and must be instructed to report to the doctor at the earliest as early recognition and discontinuation of the drug can result in good visual recovery. The visual function of first two patients recovered after the drug was discontinued. However, the third patient experience only partial recovery. This could be attributed to prolong use of the drug resulting in extensive damage to the optic nerves.

4. Conclusion

Since there is well-established evidence for Ethambutol induced toxic optic neuropathy in literature, majority of the clinicians are aware of the visual dysfunction caused by Ethambutol. However, clinicians need to be aware of sight threatening complications of Linezolid. Health care workers and patients need to be sensitized to report any visual complaints during the treatment course. Timely diagnosis and prompt intervention/stoppage of the drug is crucial for optimal visual recovery of the patients.

Conflicts of interest

The authors have none to declare.

Consent to participate/Consent for publication

(According to ICMJE Recommendations for protection of research participants): Obtained from participants.

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

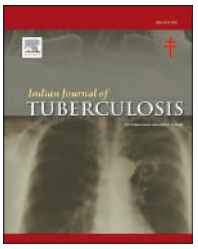
REFERENCES

1. Hashemian SMR, Farhadi T, Ganjparvar M. Linezolid: a review of its properties, function, and use in critical care. *Drug Des Dev Ther*. 2018;12:1759.
2. Singh B, Cocker D, Ryan H, Sloan DJ. Linezolid for drug-resistant pulmonary tuberculosis. *Cochrane Database Syst Rev*. 2019;3:CD012836.
3. Sharma P, Sharma R. Toxic optic neuropathy. *Indian J Ophthalmol*. 2011;59:137.
4. Lee E, Burger S, Shah J, et al. Linezolid-associated toxic optic neuropathy: a report of 2 cases. *Clin Infect Dis*. 2003;37:1389–1391.
5. Karuppannasamy D, Raghuram A, Sundar D. Linezolid-induced optic neuropathy. *Indian J Ophthalmol*. 2014;62:497–500.
6. Brandariz-Núñez D, Hernández-Corredoira V, Guarc-Prades E, García-Navarro B. Optic neuropathy associated with Linezolid: systematic review of cases. *Farmac Hosp*. 2019;43:61–65.
7. Birmingham MC, Rayner CR, Meagher AK, Flavin SM, Batts DH, Schentag JJ. Linezolid for the treatment of multidrug-resistant, gram-positive infections: experience from a compassionate-use program. *Clin Infect Dis*. 2003;36:159–168.
8. Kulkarni K, del Priore LV. Linezolid induced toxic optic neuropathy. *Br J Ophthalmol*. 2005;89:1664–1665.
9. Mehta S, Das M, Laxmeshwar C, Jonckheere S, Thi SS, Isaakidis P. Linezolid-associated optic neuropathy in drug-resistant tuberculosis patients in Mumbai, India. *PLoS One*. 2016;11(9).
10. Sotgiu G, Centis R, D'Ambrosio L, et al. efficacy, safety and tolerability of Linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. *Eur Respir J*. 2012;40:1430–1442.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Drug resistance patterns and treatment outcomes in DR-TB patients at a tertiary care centre in Mumbai

Sanchit Mohan [a,*], *Vishwanath Pujari* [b], *Priti Meshram* [c]

[a] *Grant Government Medical College, Mumbai, Flat-12, Swastik Apartment, JJ Hospital Campus, Byculla, Mumbai 400008, India*

[b] *Grant Government Medical College, Mumbai, India*

[c] *Department of Pulmonary Medicine, Grant Government Medical College and Sir JJ Group of Hospitals, Mumbai, India*

ARTICLE INFO

Article history:
Received 31 October 2022
Accepted 11 January 2023
Available online 16 January 2023

Keywords:
Drug-resistance
Tuberculosis
Pyrazinamide
Line-probe assay

ABSTRACT

Background: Drug-resistant tuberculosis (DR-TB) is a major health problem and threatens Tuberculosis (TB) control and outcomes globally. India holds one-fourth of global DR-TB burden.[1]

Aims: 1- To study drug resistance patterns and outcomes in DR-TB patients under National Tuberculosis Elimination Programme (NTEP) at a tertiary care-centre. 2- To correlate outcome of DR-TB with drug resistance patterns.

Methods: It is a retrospective study of 302 Drug Resistant Tuberculosis patients from Jan 2020 to May 2022. Common mutations of drug resistance, pyrazinamide resistance in DR-TB patients, correlation of High dose Moxifloxacin sensitivity by Line Probe Assay (LPA) and drug sensitivity test (DST), outcome of DR-TB patients with drug resistance patterns and correlation of outcome of DR-TB patients with their initial body-weight were studied.

Results: Kat G was the most common mutation in Isoniazid (96%) resistance for MDR TB as well as Isoniazid Mono-resistance TB ($p = 0.001$). 91% cases with MDR-TB were resistant to pyrazinamide. 51.2% cases had low dose Fluroquinolone resistance. 18.8% cases had low and high dose Fluroquinolone resistance. 8.5% cases had resistance to injectables. 21.7% of cases who were resistant to High dose Moxifloxacin on second line LPA were found to be sensitive on DST. Outcomes were not dependent on the LPA resistance patterns. Body-weight greater than 45 Kg at the time of initiation of treatment was associated with better outcomes ($p = 0.007$).

Conclusion: DR-TB patients are resistant to pyrazinamide in nearly all cases; hence pyrazinamide is not suitable for initial replacement sequence. Second line resistance doesn't impact outcome in DR-TB patients.

* *Corresponding author*. Flat-12, Swastik Apartment, JJ Hospital Campus, Byculla, Mumbai 400008, India. Tel.: +9561485006.
E-mail address: sanchit.agarwal1992@gmail.com (S. Mohan).

https://doi.org/10.1016/j.ijtb.2023.01.006

1. Introduction

Tuberculosis (TB) is one of the top 10 infectious diseases leading to mortality worldwide.[1] About 10 million people worldwide were infected with TB in 2018. India accounts for 27% of the total cases and 27% of the world's rifampicin-resistant cases.[1] Multi-drug resistant tuberculosis (MDR-TB) cases i.e., resistant to isoniazid and rifampicin pose a serious threat to end-TB initiative.[2] Global incidence of MDR-TB in new cases is 3.4% and in previously treated cases is 18%. National anti-tuberculosis drug resistance survey (NDRS) revealed that 28% of TB patients were resistant to at least one drug, out of which 22% were new cases and 36.8% were previously treated. Of these, 6.1% had MDR-TB. 2.8% of these MDR-TB cases were new and 11.6% previously treated.[3] MDR-TB occurs primarily due to poor adherence to TB medications, irregular use of drugs, interrupted drug supplies, physician error and accessibility of drug without prescription.[4] It is difficult to treat drug-resistant tuberculosis (DR-TB) due to higher toxicity, decreased efficacy and increased pill burden as compared to first line drugs.[5]

National tuberculosis elimination programme (NTEP) in India has developed National strategic plan (NSP) 2017–2025 to achieve the milestone of eliminating TB from India by 2025. Universal Drug susceptibility testing (UDST) and bedaquilline containing regimen has been rolled out across the country to achieve this target by 2025. This End-TB program requires further research and development as well as updated assessment of TB epidemic at regional, country and global level.

The aim of this study is to determine drug resistance patterns in DR-TB patients under NTEP at a tertiary care centre and to correlate outcome of DR-TB with drug resistance patterns.

2. Material and methods

The present study was a retrospective, observational study conducted at Nodal DR-TB centre, Grant Government Medical College, and J.J Group of Hospitals, Mumbai from Jan 2020 to May 2022. The study was approved by Institutional ethics committee.

Inclusion Criteria: All bacteriologically confirmed pulmonary or extra-pulmonary, notified DR-TB patients registered from J.J Hospital.

Exclusion Criteria: Patients who were clinically started on DR-TB regimen due to failure of first line anti-tubercular treatment were excluded from the study.

Total 302 pulmonary or extra-pulmonary TB patients who were confirmed DR-TB either by cartridge based nucleic acid amplification test (CBNAAT) or by Line probe Assay (LPA) were enrolled. First line LPA, second line LPA and drug sensitivity test (DST) was done in patients who were culture positive in Mycobacterial growth indicator tube (MGIT) under NTEP. DST was done for Pyrazinamide (PZA), Linezolid (LNZ), Moxifloxacin 1.0, Clofazimine under the programme who were resistant to either fluoroquinolone (FQ) or second line injectables (Amikacin, kanamycin) or both on second line LPA. Inh-A and Kat-G mutations were studied in first line LPA; mutation to 3A, 3B, 3C for FQ were studied in second line LPA. Correlation of High dose Moxifloxacin sensitivity by Line Probe Assay (LPA) and drug sensitivity test (DST) was also studied. Outcome was divided into positive and negative outcomes. Patients who defaulted the regimen and the patients who are still under treatment are excluded from the outcome. Only patients who successfully completed the treatment or died due to TB or its complications were included for calculating the outcome. Outcome of DR-TB patients with drug resistance patterns and correlation of outcome of DR-TB patients with their initial body-weight were studied. Initial body weight was divided into less than or equal to 45 and more than 45.

2.1. Definition

Drug-Resistant Tuberculosis (DR-TB): Resistance to any anti-tubercular drug, including acquired and primary drug resistance according to whether they had a history of previous treatment.[1,6]

Rifampicin-resistant TB (RR-TB): Resistance to rifampicin detected using phenotypic or genotypic method.[1,6]

Mono-resistant TB: Resistance to only one first line drug other than rifampicin.[1,6]

2.2. Statistical analysis

Data was analysed by SPSS-16.0 software. Chi-square test was used for various statistical comparisons. The p value < 0.05 was considered statistically significant.

3. Results

Total 302 patients were tested for rifampicin resistance by CBNAAT out of which 282 (93.4%) were found to be rifampicin-resistant and 19(0.3%) were found to be rifampicin-sensitive TB. 1 (0.3%) patient was CBNAAT negative, but came out to be positive in MGIT. (Table 1)

As per the TB treatment guidelines followed in India (PMDT), LPA is done only for patients who are either MGIT positive (Indirect LPA) or Acid-fast bacilli (AFB) smear positive (Direct LPA). First-line LPA was done in total 260 patients. Second-Line LPA was done in 204 patients (Table 2). Among isoniazid, Kat-G was the most common resistance pattern seen 180/260(69.2%) as compared to Inh-A 8/260 (3.1%), combined resistance of Kat-G and Inh-A was seen in 72/260(27.7%). Among second-line LPA, low dose fluroquinolone (FQ) resistance was the most common resistance (133/204(65.1%)), followed by high dose FQ 49/204(24%); resistance to second-line

Table 1 – Distribution of CBNAAT results of 302 patients.

Cases	Count (%)
Rifa-resistant (RR)	282 (93.4%)
Rifa-sensitive (RS)	19 (6.3%)
MTb not Detected	1 (0.3%)
Total cases	302 (100%)

Table 2 – Resistance pattern in First-line LPA and Second-line LPA.

First-line LPA n = 260	Rifampicin	Rpo	241 (92.69%)
	Isoniazid	Kat-G	180 (69.2%)
		Inh-A	8 (3.1%)
		Combined	72 (27.7%)
Second-line LPA n = 204	Fluroquinolone	Gyr-A (3A)	133 (65.1%)
		3B 3C 3D	49 (24%)
	SLID	Eis	21 (10%)
		Rrs	1 (0.04%)

Table 3 – Pattern of Isoniazid resistance in Isoniazid mono-resistant TB.

First-line LPA	Number of Cases (%) N = 19	
INH Resistant	1 (0.1%)	z-test value = 3.7613
KAT G Resistant	12 (63.1%)	p-value = 0.00016
Both resistant	6 (31.6%)	

injectables (SLID) was the least common resistance in second-line LPA: eis 21/204(10%), rrs 1/204(0.04%).

Table 3 shows 19 Isoniazid mono-resistant TB, out of which 12 were Kat-G resistant, 1 was Inh-A resistant and 6 were resistant to both Inh-A and Kat-G.

p-value for the z-test of proportion comparison of Inh-A resistant and Kat-G resistant patients indicates that the number of cases for Kat-G resistance are significantly greater than Inh-A.

137(52.6%) of RR patients were additionally resistant to either FQ or SLID. DST for PZA, Lnz, Clofazimine, Moxifloxacin (1.0 mcg/dl) is done only for patients who have additional resistance in second-line LPA. It was seen that 125(91.2%) patients were resistant to PZA (Table 3).

p-value less than that of 0.05 indicates that the resistance is significantly present in high-dose Moxifloxacin(1.0 mcg/dl) on DST, out of the total high dose moxifloxacin resistance on LPA.

Out of 302, only 139 patients completed treatment, 28 defaulted the treatment. Patients who defaulted the regimen were excluded in calculating the outcome. Patients who completed the regimen and were disease-free, were declared as cured; patients who died or culture positive even after 1 year of treatment were declared as failed.

Table 5 shows 66.7% patients who were resistant to all drugs on LPA, 46.7% patients who were sensitive to all drugs on LPA and 54.7% who were resistant to at least one drug on

Table 5 – Impact of LPA patterns of resistanceand initial body-weight on treatment outcomes.

LPA results	Final treatment outcome	
	Cured (%)	Failed (%)
All Sensitive	21 (46.7%)	24 (53.3%)
All Resistant	14 (66.7%)	7 (33.3%)
At least one Resistant	41 (54.7%)	34 (45.3%)
LPA High dose FQ (3B, 3C, 3D) (n = 120)		
Resistant	16 (25.8%)	15 (25.9%)
Sensitive	46 (74.2%)	43 (74.1%)
	p = 0.994	
Weight (n = 139)		
≤45	32 (43.2%)	42 (56.8%)
>45	43 (66.2%)	22 (38.8%)
	p = 0.007	

LPA were cured. There was no significant difference between treatment outcome and resistance pattern on LPA.

Significance of high dose FQ resistance to treatment outcome was also calculated. p-value for the chi-square was greater than that of 0.05, indicating no significant association between resistance of high dose FQ on LPA (FQ 3B 3C 3D) and the final treatment outcome.

Final treatment outcome was also compared with initial body weight at the time of treatment initiation. Body weight was divided into less than or equal to 45 kg and more than 45 kg. 74 patients were less than or equal to 45 kg and 65 patients were more than 45 kg at the time of treatment initiation. p-value less than 0.05 indicates that higher weight is associated with good treatment outcome.

4. Discussion

CBNAAT was done for all patients to look for drug resistance. Total 302 patients were enrolled, out of which 282 (93.4%) were found to be rifampicin-resistant and 19 (0.3%) were found to be rifampicin-sensitive TB. 1 (0.3%) patient was CBNAAT negative, but came out to be positive on MGIT (Table 1).

First-line LPA was done in total 260 patients (Table 2). Among isoniazid, Kat-G was the most common resistance pattern seen 180/260 (69.2%) as compared to Inh-A 8/260 (3.1%); combined resistance of Kat-G and Inh-A was seen in 72/260 (27.7%). Senia Rosales-Klintz et al in a study from Belarus, China, Iran/Iraq, Honduras, Romania, and Uganda showed that 82% MDR patients with Isoniazid resistance had Kat-G mutation.[7] Giri Prasad Polu et al in a study conducted in southern coastal region of Andhra Pradesh, showed results in

Table 4 – PZA resistance among MDR-TB patients and genotype-phenotype correlation of moxifloxacin resistance.

DST for PZA n = 137 (%)		DST for Moxifloxacin (1.0) out of SLLPA showing resistance to high dose Moxifloxacin n = 46 (%)	
Resistant	Sensitive	Resistant	Sensitive
125 (91.2%)	12 (9.8%)	36 (78.3%) p = 0.0004	10 (21.7%)

concordance to present study 75.25% had mutation in Kat-G and 24.75% had mutation in inh-A gene, however they could not find combined resistance in Inh-A and Kat-G.[8] R.N. Yadav et al conducted a study at AIIMS New Delhi showed frequencies of resistance in Kat-g, Inh-A, Combined as 72/87 (83%), 10/87 (11%) and 5/87 (6%) respectively.[9] Combined resistance in present study was quite higher as compared to all previous studies conducted. Among 19 cases who were mono-resistant to Isoniazid (Table 3), 1/19 (0.1%) was resistant to Inh-A, 12/19 (63.1%) were resistant to Kat-g and 6/19 (31/6%) were resistant to both Inh-A and Kat-g. It was seen that Kat-G was significantly common mutation as compared to Inh-A ($p = 0.00016$).

Patients in present study were evaluated for frequency in gyr-A mutation. Mutation in gyr-A from 1 to 3A were classified as low level FQ resistance and any mutation in 3B, 3C or 3D was classified as high level FQ resistance.[1] Among second-line LPA, low dose fluroquinolone (FQ) resistance was the most common resistance 133/204 (65.1%), followed by high dose FQ 49/204 (24%). Resistance to second-line injectables (SLID) was the least common resistance in second-line LPA i.e., eis 21/204 (10%), rrs 1/204 (0.04%). In a meta-analysis by Dennis Falzon et al, in a study conducted in Canada showed 4763 (71%) patients had MDR-TB but were susceptible to both fluoroquinolones and second-line injectable drugs (MDR-TB only), 1130 (17%) had MDR-TB + INJr, 426 (6%) had MDR-TB + FQr and 405 (6%) had XDR-TB.[10]

Pyrazinamide (PZA) is an essential drug and comes under the initial replacing sequence of second line TB drugs after Delaminid and second-line injectables.[1] DST for PZA is done under the programme (NTEP), if there is resistance in second line LPA. In the present study, DST for PZA was available for 137 patients, out of which 125 (91.2%) patients were resistant to PZA. Whitfield MG et al conducted a meta-analysis and showed PZA resistance was 60.5% in MDR-TB patients, 41.3% in TB patients at high-risk of MDR-TB.[11] Y. Che et al in their study conducted in China showed that the PZA resistance rate among MDR-TB in Ningbo was 59.1%.[12]

Patients who were resistant to high dose FQ on LPA (3B,3C, 3D mutation) and whose DST for Moxifloxacin (1.0 mcg/dl) was available, were tested for discordance between high dose FQ resistance on LPA and DST (Table 4). 36/46 (78.3%) patients were also resistant on DST and 10/46 (21.7%) patients were sensitive on DST ($p = 0.0004$). p-value less than that of 0.05 indicates that the resistance is significantly present in High dose Moxifloxacin (1.0 mcg/dl) on DST, out of the total high dose moxifloxacin resistance on LPA. Agrawal U et al studied specific FQ mutations in LPA predicting susceptibility in DST at critical concentrations of moxifloxacin at 0.5 and 2 mcg/dl at a tertiary care centre in Mumbai and found that 49/59 (83.1%) patients were sensitive to high dose moxifloxacin on DST despite of resistance in LPA (MUT 3B,3C,3D).[13] Yadav RN et al studied 46 high dose moxifloxacin resistant cases on LPA to look for concordance on DST moxifloxacin (1.0 mcg/dl) and found that 36 (78%) cases were resistant and 10 (22%) cases were sensitive.[14]

LPA resistance pattern was compared with final outcome. It was seen that 66.7% patients who were resistant to all drugs on LPA, 46.7% patients who were sensitive to all drugs on LPA and 54.7% who were resistant to at least one drug on LPA, were cured. There was no significant difference between treatment outcome and resistance pattern on LPA (Table 5). In contrast to this, study by Nishtha et al conducted a study at King George Medical University, Lucknow and showed that second-line drug resistance, especially to FQ, was significantly associated with unfavourable treatment outcomes ($p = 0.001$).[15]

Present study shows there is no significant association between treatment outcome and high dose FQ resistance on LPA (MUT 3A, 3B, 3D) ($p = 0.992$). This was in discordance to the study conducted by L. Rigouts et al, in a study conducted in Bangladesh showed that gyr-A mutation in 3B, 3C, 3D resulted in only 30.8% cure rate, whereas all other gyr-A mutation resulted in 65.6% cure rate ($p = 0.008$).[16] Maha R Farhatet et al in a study conducted at Lima, also showed that high level gyr-A mutation significantly predicts poorer outcomes with a hazard ratio of 2.6 (1.2–5.6).[17]

Patients who weighed more than 45 kg at the time of treatment initiation were associated with significantly better treatment outcome ($p = 0.007$). Imran khan et al conducted similar study in Pakistan and had outcomes in concordance to present study that body weight <40 kg had statistically significant negative association with treatment failure.[18]

5. Conclusion

Kat-G is the most common mutation for resistance of isoniazid followed by combined mutation of Kat-g and Inh-A. PZA comes as a 3rd drug in replacement sequence after Delaminid and Amikacin. DST for PZA is not always present under the NTEP programme. Present study proves significant number (91.2%) of DR-TB cases are resistant to PZA. There is discordance between resistance of high dose FQ on LPA and DST though the result is insignificant. However, 21.7% patients were sensitive on DST despite of resistance on LPA. There is no significant association between resistance on second-line LPA and treatment outcome. High dose moxifloxacin resistance is not associated with negative outcomes. Body weight <45 at time of diagnosis had significant association with poorer outcomes.

6. Limitations

LPA/DST were not available for each and every patient as this study was conducted as per the management norms of NTEP hence the sample size was less.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. World Health Organization. *Global Tuberculosis Report 2019*. Geneva: World Health Organization; 2019. Available from: http://www.who.int/tb/publications/global_report/en. Accessed October 19, 2020.
2. Ullah I, Javaid A, Tahir Z, et al. Pattern of drug resistance and risk factors associated with development of drug resistant *Mycobacterium tuberculosis* in Pakistan. *PLoS One*. 2016;11: e0147529. https://doi.org/10.1371/journal.pone.0147529.
3. Directorate of Health Services, Ministry of Health & Family Welfare, Government of India CTD. *National Anti-TB Drug Resistance*; 2018. Survey.pdf [Internet]. https://tbcindia.gov. in/showfile.php?lid=3315. Accessed March 16, 2021
4. Range N, Friis H, Mfaume S, et al. Anti-tuberculosis drug resistance pattern among pulmonary tuberculosis patients with or without HIV infection in mwanza, Tanzania. *Tanzan J Health Res*. 2012:1–6.
5. Sbammpsc NC. Pattern of drug-resistance and treatment outcome in multidrug-resistant pulmonary tuberculosis. *Indian Journal of Chest Diseases & Allied Sciences*. 2012:23–26.
6. Van RA, Warren R, Richardson M, et al. Classification of drug-resistant tuberculosis in an epidemic area. *Lancet*. 2000;356(9223):22–25. https://doi.org/10.1016/s0140-6736(00)02429-6.
7. Rosales-Klintz Senia, Jureen Pontus, Zalutskayae Aksana, et al. Drug resistance-related mutations in multidrug-resistant Mycobacterium tuberculosis isolates from diverse geographical regions. *International Journal of Mycobacteriology*. 2012;1(3):124–130. https://doi.org/10.1016/j.ijmyco.2012.08.001. ISSN 2212-5531.
8. Polu Giri Prasad, Mohammad Shaik Jasmine, Mani kanta Kota Neela, Karumanchi Deepthi, Allam Uday Sankar. Analysis of drug resistance mutations in pulmonary Mycobacterium tuberculosis isolates in the Southern coastal region of Andhra Pradesh, India. *Braz J Infect Dis*. 2019;23(5):281–290. https://doi.org/10.1016/j.bjid.2019.07.002. ISSN 1413-8670.
9. Yadav RN, Singh BK, Sharma SK, et al. Comparative evaluation of GenoType MTBDRplus line probe assay with solid culture method in early diagnosis of multidrug resistant tuberculosis (MDR-TB) at a tertiary care centre in India. *PLoS One*. 2013;8. Article e72036.
10. Falzon D, Gandhi N, Migliori GB, et al. Collaborative Group for Meta-Analysis of Individual Patient Data in MDR-TB. Resistance to fluoroquinolones and second-line injectable drugs: impact on multidrug-resistant TB outcomes. *Eur Respir J*. 2013 Jul;42(1):156–168. https://doi.org/10.1183/09031936.00134712. Epub 2012 Oct 25. PMID: 23100499; PMCID: PMC4487776.
11. Whitfield MG, Soeters HM, Warren RM, et al. A global perspective on pyrazinamide resistance: systematic review and meta-analysis. *PLoS One*. 2015;10(7), e0133869. https://doi.org/10.1371/journal.pone.0133869.
12. Che Y, Bo D, Lin X, et al. Phenotypic and molecular characterization of pyrazinamide resistance among multidrug-resistant *Mycobacterium tuberculosis* isolates in Ningbo, China. *BMC Infect Dis*. 2021;21:605. https://doi.org/10.1186/s12879-021-06306-1.
13. Agrawal U, Savaj P, Davda K, Rodrigues C, Soman R. Correlation of specific mutations in line probe assay (LPA) and drug susceptibility test (DST) with respect to fluoroquinolone resistance in drug resistant *Mycobacterium tuberculosis*. *Open Forum Infect Dis*. 2017 Oct 4;4(suppl 1):S619–S620. https://doi.org/10.1093/ofid/ofx163.1637. PMCID: PMC5630702.
14. Yadav RN, Bhalla M, Kumar G, Sah GC, Dewan RK, Singhal R. Diagnostic utility of GenoType MTBDR*sl* assay for the detection of moxifloxacin-resistant *mycobacterium tuberculosis*, as compared to phenotypic method and whole-genome sequencing. *Int J Mycobacteriol*. 2022;11:183–189.
15. Singh Nishtha, Singh Pravin Kumar, Singh Urmila, Garg Rajiv, Jain Amita. Fluroquinolone drug resistance among MDR-TB patients increases the risk of unfavourable interim microbiological treatment outcome: an observational study. *Journal of Global Antimicrobial Resistance*. 2021;24:40–44. https://doi.org/10.1016/j.jgar.2020.11.011. ISSN 2213-7165.
16. Rigouts L, Coeck N, Gumusboga M, et al. Specific *gyrA* gene mutations predict poor treatment outcome in MDR-TB. *J Antimicrob Chemother*. February 2016;71(2):314–323. https://doi.org/10.1093/jac/dkv360.
17. R Farhat Maha, Jacobson Karen R, Franke Molly F, Kaur Devinder, Murray Megan, Mitnick Carole D. Fluoroquinolone resistance mutation detection is equivalent to culture-based drug sensitivity testing for predicting multidrug-resistant tuberculosis treatment outcome: a retrospective cohort study. *Clin Infect Dis*. 15 October 2017;65(8):1364–1370. https://doi.org/10.1093/cid/cix556.
18. Khan Imran, Ahmad Nafees, Khan Shereen, et al. Evaluation of treatment outcomes and factors associated with unsuccessful outcomes in multidrug resistant tuberculosis patients in Baluchistan province of Pakistan. *Journal of Infection and Public Health*. 2019;12(6):809–815. https://doi.org/10.1016/j.jiph.2019.04.009. ISSN 1876-0341.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Original article

Strain identification of *Mycobacterium tuberculosis* in multidrug-resistant tuberculosis (MDR-TB) patients at Undata Hospital, Palu, Central Sulawesi

Gunawan [a,*], *Meiske Elisabeth Koraag* [a], *Muchlis Syahnuddin* [a], *Afi Nursafingi* [a], *Made Agus Nurjana* [a], *Phetisya P.F. Sumolang* [b], *Murni* [b], *Nurul Hidayah* [b], *Irawati* [b]

[a] *Research Center for Public Health and Nutrition, Research Organization for Health, National Research and Innovation Agency, Indonesia*

[b] *Donggala Health Research and Development Center, Ministry of Health RI, Indonesia*

ARTICLE INFO

Article history:
Received 8 August 2022
Received in revised form
30 March 2023
Accepted 5 April 2023
Available online 18 April 2023

Keywords:
MDR TB
Spoligotyping
Mycobacterium tuberculosis
Undata Hospital
Palu

ABSTRACT

MDR-TB is a tuberculosis disease resistant to the two most effective anti-TB drugs, rifampin and isoniazid. MDR-TB is a threat to TB control. This study aims to identify the *Mycobacterium tuberculosis* strain in MDR-TB patients at Undata Hospital in Palu, Central Sulawesi. This type of research is descriptive and observational with a cross-sectional design. The study was conducted in Palu City, Donggala Regency, and Sigi Regency from April–June 2021. The sample in this study consisted of 22 patients who had undergone MDR-TB treatment from 2019 to 2020. The results showed that 55% of the patients with MDR-TB lived in Palu City, 27% in Donggala Regency, and 18% in Sigi Regency. Out of the 22 patients, 13 were men (59%) and nine were women (41%). Based on the results of the examination of *Mycobacterium tuberculosis* culture, three samples from patients with MDR-TB were positive for *M. tuberculosis*. The results of the spoligotyping examination showed that the strain belonged to the Beijing family. Identifying the type of *M. tuberculosis* strain through spoligotyping examination should be carried out in TB patients who have not undergone MDR-TB treatment or those who have failed treatment and are still found to be positive for *M. tuberculosis* bacteria.

1. Introduction

Drug-resistant tuberculosis (TB) is a major public health problem and a significant threat to global TB control.[1] Traditionally, drug resistance in TB has been classified into three types: primary drug resistance, acquired drug resistance, and initial drug resistance. Primary drug resistance occurs when previously untreated patients are diagnosed with drug-resistant organisms, likely because they have been

* *Corresponding author*.
E-mail address: guna017@brin.go.id (Gunawan).
https://doi.org/10.1016/j.ijtb.2023.04.003

infected from an outside source of resistant bacteria. This type of drug resistance is uncommon in Canadian-born individuals, unless they have travelled to a country with a high prevalence of anti-TB drug resistance. The second type, acquired drug resistance, occurs when patients who initially have drug-susceptible TB bacteria later become drug-resistant due to inadequate, inappropriate, or irregular treatment or non-adherence to drug-taking. This type of drug resistance is also uncommon in Canadian-born individuals, as directly observed therapy is frequently used to promote treatment adherence. The third type of drug resistance is initial drug resistance, which occurs in patients who deny previous treatment but whose history of prior drug use cannot be verified. In reality, this type of resistance consists of true primary resistance and an unknown amount of undisclosed acquired resistance. These patients are classified as having initial rather than primary drug resistance.[2]

One of the major challenges facing the global community is the growing number of patients with multidrug-resistant tuberculosis (MDR TB). In 2012, there were an estimated 440,000 new cases of MDR TB worldwide, resulting in around 150,000 deaths. The number of new cases increased in 2013, with an estimated 480,000 cases and 210,000 deaths reported. In the same year, MDR TB accounted for 5% of all TB cases, with 3.5% of cases being newly diagnosed and 20.5% occurring during treatment. According to the World Health Organization (WHO), there were as many as 558,000 MDR/RR TB cases globally in 2017, with 82% (460,000–560,000) being MDR TB. The number of deaths due to MDR/RR TB in 2017 was around 230,000 (range, 140,000–310,000). China, India, and the Russian Federation are the countries with the highest number of MDR/RR TB cases, accounting for 47% of cases worldwide. Indonesia ranks seventh globally, with 23,000 incident cases reported in 2017 (range, 16,000–31,000), after Nigeria which had more cases.[3]

In recent years, molecular biology technology has advanced significantly, enabling the identification and differentiation of various strains of *Mycobacterium tuberculosis* in TB patients. One method used for this purpose is the Spacer Oligonucleotide Typing (Spoligotyping) technique.[4] Spoligotyping is a molecular technique that uses DNA hybridization to identify *M. tuberculosis* strains based on the presence or absence of spacer regions found in direct repeats (DR) in the *M. tuberculosis* genome. This region consists of 43 iterations, each consisting of 36 base pairs, with each loop separated by a spacer that can be detected by the Spoligotyping method to distinguish between different *M. tuberculosis* strains. Spoligotyping can effectively show the division of specific strains and is particularly useful in distinguishing the Beijing strain group from others. The Beijing strain is characterized by the absence of hybridization in spacers 1–34 and hybridization in at least 3 of the last nine spacers, making it easily identifiable through Spoligotyping.[5,6]

Research on the molecular epidemiology of TB in Indonesia was conducted by Lisdawati et al.[7] The study found that most *M. tuberculosis* genotypes in Indonesia were of the Beijing type, based on samples from Java, Sumatra, and Kalimantan. Meanwhile, types EAI (East Africa Indian) and LAM (Latin American Mediterranean) dominated eastern Indonesia.[8] The study also established a link between the *M. tuberculosis* genotype and susceptibility to resistance to OAT. The Beijing strain showed the highest susceptibility to OAT resistanceity to resistance to OAT. The highest OAT resistance susceptibility was found in the Beijing strain.

Spoligotyping has been widely used to trace transmission of infectious diseases because it is a simple, fast, and reliable method for simultaneously detecting them.[4] However, despite the significant prevalence of TB in Indonesia, it is still unknown which strain of *M. tuberculosis* is the most dominant in infecting most TB patients in each province.[9] This information is crucial since different strains have different characteristics, such as resistance to antibiotics. Thus, identifying the dominant strain that infects TB patients in a particular area can facilitate therapy and prevent transmission. According to reported research, the Beijing genotype is the most dominant strain of *M. tuberculosis* in several Southeast Asian countries, such as China, Korea, and Hong Kong, accounting for 86% of cases.[10]

The national TB control program has not implemented TB surveillance in Central Sulawesi using biomolecular methods. Therefore, researchers will conduct a study on identifying the *M. tuberculosis* strain in patients with Multiple Drug Resistance Tuberculosis (MDR TB) at Undata Hospital Palu, Central Sulawesi. The objective of this study is to identify the *M. tuberculosis* strain in MDR TB patients at Undata Hospital Palu using the spoligotyping method. The novelty of this research lies in the original research conducted in this specific region and the use of spoligotyping to identify *M. tuberculosis* strains.

2. Materials and methods

This research has received approval from the Research Ethics Commission of the Faculty of Medicine, Tadulako University, with the number 9013/UN 28.1.30/KL/2020. The type of research conducted is descriptive and observational with a cross-sectional design. The study was carried out in Palu City, Donggala Regency, and Sigi Regency from April to June 2021. The study population consisted of MDR TB patients at Undata Hospital whose medical records and primary data forms for suspected MDR TB patients, MDR TB registers 03, and the TB.06 MDR form were used for sampling. The population studied included MDR TB patients living in Palu City, Donggala Regency, and Sigi Regency in 2019–2020.

Sputum collection from MDR TB patients was performed three times within two days using the SPS method, once in the morning and twice on the second day, once in the morning and again after collection in the morning. Patients were advised to expel phlegm on an empty stomach. Sterile sputum pots were labeled with the patient's name and the date of sputum collection. The sputum was then placed in sealed plastic bags, with one bag containing one phlegm pot. The sputum pots were sealed with parafilm and stored at room temperature for 48 h. If the storage time was between 48 and 72 h, the sputum sample was placed in a styrofoam box with an ice pack at a temperature of 4–8 °C. The next step was to send the samples to the Microbiology Laboratory at Padjadjaran University in Bandung.

The culture of *M. tuberculosis* was carried out in the BSL-3 Microbiology Laboratory, Padjadjaran University. The

sputum samples from MDR TB patients were decontaminated using the 2% NaOH method and then inoculated into Ogawa medium and the Mycobacterium Growth Indicator Tube (MGIT), which had been supplemented with Oleic Albumin Dextrose Catalase (OADC) and the antibiotic complex Polymyxin, Azlocillin, Nalidixic acid, Trimethoprim, and Amphotericin B (PANTA)® (Becton Dickinson).[6]

The culture was incubated at 37 °C for 14–21 days. Bacteria cultured on Ogawa media were used for spoligotyping purposes. Identification of *M. tuberculosis* was carried out by observing the colony morphology, and colonies with a characteristic pale white color and complex appearance on Ogawa media were identified as *M. tuberculosis*[6].

The extraction of *M. tuberculosis* DNA was performed using the QIAamp Mini Kit (Qiagen, Germany) following the manual procedure with modifications. Once all *M. tuberculosis* samples had grown on Ogawa's medium, the following procedure was used for DNA extraction. Colonies that grew on Ogawa solid media were taken and placed in an Eppendorf tube containing 200 μl of Phosphate Buffer Saline (PBS). The colony pellet was then crushed using a sonicator for 1 min, followed by DNA extraction. A Spoligotyping examination was performed using a spoligotyping kit (Ocimum®, Biosplution < Hyderabad, India). The extracted DNA product was then amplified on the Direct Repeat (DR) gene fragment using the primers available in the kit, namely Dra (5′GGTTTTGGGTCTGACGAC3′) and DRb (5′CCGAGAGGGGACGGAAAC3′). The composition of the PCR reaction was 2 μl DNA template, 4 μl DRa primer (20 pmol), 4 μl DRb primer (20 pmol), 4 μl dNTP, 5 μl 10x buffer Super T (Ocimum), 0.1 μl Super T polymerase (five units/μl) (Ocimum) and MQ water (Ocimum) up to a total volume of 50 μl.[6]

DNA amplification was carried out under pre-denaturation conditions of 96 °C for 3 min, followed by denaturation at 96 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 30 s, for 20 cycles, ending with a final elongation at room temperature at 72 °C for 5 min. The mini blotter was prepared by placing the membrane and support cushion. The PCR product (20 μl) was mixed with 150 μl of 2x SSPE/0.1% SDS (Ocimum) and then pipetted into the mini blotter slot. The hybridization process was carried out for 60 min at 60 °C in an incubator oven. The membrane was then removed using tweezers, and the sample was aspirated from the mini blotter. The membrane was washed with 250 μl of 2x saline-sodium phosphate-EDTA (SSPE)/0.5% sodium dodecyl sulfate (SDS) (Ocimum) for 10 min at 45 °C, followed by two rinses with 250 μl of 2x SSPE/0.5% SDS at room temperature for 5 min each. Finally, the membrane was immersed in 20 ml of Enhanced Chemiluminescence (ECL) (Ocimum®) for 1 min while placed in plastic. The hybridization pattern was read in a dark room by attaching the hyper x-ray film to the membrane for 20–30 min.[6]

The univariate analysis was conducted to determine the characteristics of the contact cases. The reading of the hybridization pattern to determine the type of *M. tuberculosis* strain was done manually by comparing the spoligotyping pattern with the Spoligotyping Database map.

Table 1 – The characteristics of MDR TB patients at Undata Hospital Palu.

Characteristics	Quantity (N:22)
Domicile	
Palu City	12 (55.0)
Regency. Donggala	6 (27)
Regency. Sigi	4 (18)
Age (%)	
17–25	3 (14.0)
26–45	8 (36.0)
46–65	11 (50.0)
Gender (%)	
Male	13 (59.0)
Female	9 (41.0)

3. Results and discussion

3.1. Characteristics of MDR TB Patients at Undata Hospital Palu

A total of 22 MDR TB patients were included in this study, and their records were collected from the medical records of MDR TB suspected patients, primary data forms for suspected MDR TB patients, MDR TB.03 registers, and MDR TB.06 forms at the Undata Hospital. The study samples were MDR TB patients who resided in Palu City, Donggala Regency, and Sigi Regency. The characteristics of the research subjects are presented in Table 1.

The results of the study revealed that the majority of MDR TB patients were from Palu City, the capital of Central Sulawesi Province. Palu City comprises of 8 sub-districts and 46 urban villages with a population of 371,365 individuals and a population density of 940 people/km^2, according to the Central Statistics Agency of Palu City.[11] Donggala Regency, on the other hand, is composed of 16 sub-districts, nine sub-districts, and 158 villages with a population of 304,110 individuals and a population density of 58 people/km^2, according to the Central Statistics Agency of Donggala Regency.[12] Meanwhile, Sigi Regency encompasses 15 sub-districts and 177 villages with a population of 239,421 individuals and a density level of 46 people/km^2.[13]

The number and distribution of the population greatly determine the population density in an area. Population density can determine how quickly a disease spreads or becomes contagious.[14] This means that the risk factor for the spread of pulmonary TB disease caused by the bacteria *M. tuberculosis* can spread through the air, so the condition of a densely populated area is one of the factors that can accelerate the transmission of pulmonary TB. Additionally, socioeconomic conditions, poor environment, and poor nutritional conditions also contribute to this. These factors create a slum-like impression, making it easier for pulmonary TB disease to spread.[15]

According to the WHO in Aditama, areas with high population density tend to have slum dwellings and poor hygiene and nutrition, so if residents are affected by tuberculosis, it will accelerate the spread of the disease.[15] Based on this, it shows that the level of population density has a significant role in increasing the spread of MDR TB. The same with research conducted in the City of Banjarmasin by Arifin et al.[16] that a very dense environment facilitates the spread of TB and plays an essential role in increasing the number of TB cases.[16]

The characteristics of MDR TB patients show that most sufferers come from Palu City, with the highest age range being 46–65 years, and a male gender predominance. This is similar to the research conducted by Pratama et al.[24] on MDR TB patients at Sanglah Hospital, Denpasar, where the majority of patients were of productive age. A study by Surkova et al.[17] also showed that the majority of MDR TB patients in Belarus and Iran were patients of productive age.[17] This is consistent with the research conducted by Tao et al., which found that patients aged 46–65 years occupied the highest position among MDR TB patients.[18] Patients of productive age generally have high levels of daily activities, which can lead to forgetfulness in taking medication regularly.[19] Productive age is associated with a higher risk of experiencing MDR TB due to their increased activity levels, in addition to the possibility of inappropriate or incomplete treatment in the past.[20]

The predominance of male MDR TB patients in this study, with a total of 13 patients (59.0%), is consistent with research conducted by Tao et al.[21]; which found that 55% of MDR TB patients in Namibia were male.[21] This gender disparity may be due to the higher prevalence of MDR TB in men, heavier workloads, lack of rest, unhealthy lifestyles such as smoking and alcohol consumption, differences in outdoor activities, particularly work-related activities, and exposure to air pollution.[22] Men often work outside the home, exposing them to a higher risk of contact with the environment where TB is a transmission source. The prevalence of pulmonary TB is similar between men and women until adolescence, but after that, the prevalence in men is higher than in women. This is likely because contact is limited to a smaller environment until adolescence. After reaching adulthood, men have more contact with the environment outside the home than women, in addition to biological and socio-cultural factors, including TB stigma.[3]

3.2. Mycobacterium tuberculosis *culture examination and spoligotyping examination*

A culture examination of *Mycobacterium tuberculosis* was performed on 22 sputum samples from MDR TB patients. *M. tuberculosis* culture examination and Spoligotyping examination are presented in Table 2.

Based on the results of the spoligotyping examination, three sputum samples from MDR TB patients from Undata Hospital were classified as the *M. tuberculosis* strain Beijing. This is consistent with research conducted by Lisdawati et al.[7]; which found that the western and central parts of Indonesia tended to be exposed to the *M. tuberculosis* strain Beijing compared to eastern Indonesia, based on spoligo genotype data (spolDB4).[8] Similarly, Yudani and Austin (2014) found that 9 out of 29 MDR TB patients treated as outpatients at the Lung Poly RSSA Malang were genotyped as Beijing families.[4] However, this finding is very different from what was found in West Timor Province, where the Beijing strain did not dominate the isolates found, and most (82.7%) of the TB strains in Jayapura City were off the Non-Beijing type.[6,9,23]

Table 2 – Examination of *Mycobacterium tuberculosis* culture and spoligotyping examination.

Checking type	Number of Samples	Result	
		Positive	Negative
Mycobacterium culture	22	3	19
Spoligotyping[a]	3	W-Beijing genotype	

[a] Note. The examination is only carried out on samples that are declared "positive".

The Beijing strain is a significant part of the Asian *M. tuberculosis* phylogenetic lineage. The Beijing strain represents about 50% of all TB strains in East Asia and at least 13% of strains worldwide. The Beijing strain of *M. tuberculosis* is predicted to cause increased bacterial virulence, resistance to treatment, and contribute to treatment failure.[2] The Beijing strain is widely distributed in Asian, African, European, Australian, American, and Latin American countries.[6]

The limitation of this study is the small sample size, which is due to the limited availability of MDR-TB patients in the hospital, time and resource constraints, and difficulty in obtaining consent from patients to participate in the study. The study only includes MDR-TB patients who are undergoing treatment, which may exclude other groups of patients and lead to selection bias. This research was conducted in only one hospital, which may not be representative of MDR-TB patients in other regions.

The sample in this study consisted of MDR TB patients undergoing treatment. Therefore, it was highly likely that the results of the bacterial culture examination would give a negative result for *M. tuberculosis*. Identification of the type of *M. tuberculosis* strain through spoligotyping examination should be carried out on TB patients who have not undergone MDR TB treatment or those who have failed treatment and are still found to be positive for *M. tuberculosis* bacteria.

4. Conclusion

The molecular identification of *M. tuberculosis* strains is Beijing genotype in all positive samples for *M. tuberculosis*.

Conflict of interest

The authors declare no potential conflict of interest in writing this article.

Acknowledgements

The authors would like to express gratitude to Donggala Health Research and Development Center, Ministry of Health RI.

REFERENCES

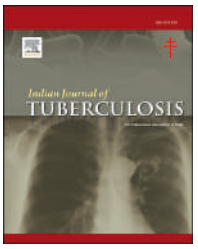
1. Indonesia Ministry of Health. *Indonesia Health Profile*. 2018. Jakarta.

2. Mertaniasih Ni Made. Acid-fast bacilli conversion of Beijing and non-Beijing strain of pulmonary tuberculosis in south Sulawesi. *Indonesian Journal of Tropical and Infectious Disease*. 2019;7(5):99–104. https://doi.org/10.20473/ijtid.v7i5.9670.
3. World Health Organization (WHO). *Global Tuberculosis Report 2021*. World Health Organization, Regional Office for Sout-East Asia; 2021.
4. Yudani T, Astuti T. The distribution of M. Beijing Genotype Tuberculosis in Pulmonary Tuberculosis Patients in Malang. *Medical Journal of Brawijaya University*. 2014;26(2):69–74. https://doi.org/10.21776/ub.jkb.2010.026.02.3.
5. David S, Raposo D, Antunes A, et al. Contribution of Spoligotyping to the characterization of the population structure of Mycobacterium tuberculosis isolates in Portugal. *Infect Genet Evol*. 2007;7:609–617. https://doi.org/10.1016/j.meegid.2007.05.007.
6. Krismawati H, Oktavian A, Chaidir L, et al. Spoligotyping, resistance of Mycobacterium tuberculosis to TB drugs and risk factors for transmission of pulmonary tuberculosis in Jayapura city. *Health Research Bulletin*. 2019 March;47(1):1–12. https://doi.org/10.22435/bpk.v47il.18.
7. Lisdawati V, Parwati I, Sudarmono P, Sudiro M, et al. Preliminary mapping study of Mycobacterium tuberculosis complex DNA by spoligotyping on sputum isolation results of pulmonary tuberculosis patients. *Health Research Bulletin*. 2010;38(4):208–224.
8. Lisdawati V, Parwati I, Sudarmono P, Sudiro TM, et al. Preliminary mapping study of Mycobacterium tuberculosis complex DNA by spoligotyping on sputum isolation results of pulmonary tuberculosis patients from 10 provincial capitals. *Health Research Bulletin*. 2010;38(4(Part I)):208–224.
9. Parvati I, Crevel Reinout V, Sudiro Mirawati, et al. Mycobacterium tuberculosis population structures differ significantly on two Indonesian Islands. *J Clin Microbiol*. 2008;46(11):3639–3645. https://doi.org/10.1128/JCM.00605-08.
10. Toungoussova OS, Sandven Per, Mariandyshev Andrey O, et al. Spread of drug-resistant Mycobacterium tuberculosis strains of the Beijing genotype in the Archangel Oblast, Russia. *J Clin Microbiol*. 2002;40(6):1930–1937. https://doi.org/10.1128/JCM.40.6.11930.
11. Palu City Statistics Center. *Palu City in 2021 Figures*. Palu, Indonesia: City Statistics Center; 2021.
12. Donggala Regency Central Statistics Agency. *Donggala Regency in 2021 Figures*. Donggala, Indonesia: Donggala Regency Central Statistics Center; 2021.
13. Sigi Regency Central Statistics Agency. *Sigi Regency in 2021 Figures*. Sigi, Indonesia: Sigi Regency Central Statistics Agency; 2021.
14. Rohman H, Janah Hanifah M, Muharry Andi. Spatial pattern pulmonary tuberculosis patient: accessibility, environment and life style factor in rural area. In: *Proceeding International Seminar on Rural, Urban and Community Health (ISRUNCH) 2018: innovative health care to improve public health in rural and urban areas*. 2018:8–16. ISBN: 978-602-53927-0-2.
15. Aditama RTY, Suharyo S. Analysis of distribution and risk factors of pulmonary tuberculosis through mapping by region at Candilama Health Center Semarang in 2012. *Journal of Health Udinus*. 2012;4(1):19–23.
16. Arifin S, et al. Implementation of the hospital development program for accelerating the health status of TN children. *Proceedings of the National Seminar on Community Service*. 2020;2(2015):43–51.
17. Surkova L, Horevich HL, Titov LP, et al. A study on demographic characteristics of drug-resistant Mycobacterium tuberculosis isolates in Belarus. *International Journal of Mycobacteriology*. Asian-African Society for Mycobacteriology. 2012;1(2):75–81. https://doi.org/10.1016/j.ijmyco.2012.04.001.
18. Lu Z, Jiang w, Zhang J, et al. Drug resistance and epidemiology characteristics of multidrug-resistant tuberculosis patients in 17 provinces of China. *PLoS Neglected Tropical Diseases*. 2019 November;2:1–14. https://doi.org/10.1371/journal.pone.0225361.
19. Bawonte TG, Mambo CD, Masengi ASR. Factors affecting tuberculosis multidrug resistance (MDR TB). *Biomedicine*. 2021;9(1):117–125.
20. Kawatsu L, Uchimura K, Izumi K, et al. Treatment outcome of multidrug-resistant tuberculosis in Japan – the first cross-sectional study of Japan tuberculosis surveillance data. *BMC Infectious Disease*. 2018;18(445):1–7. https://doi.org/10.1186/s12879-018-3353-9.
21. Ricks PM, Mavhunga F, Modi Surbhi, et al. Characteristics of multidrug-resistant tuberculosis in Namibia. *BMC Infectious Diseases*. 2012;12(385):1–8. https://doi.org/10.1186/1471-2334-12-385.
22. Nunkaidah M, Lestari H, Afa JR. The risk prevalence of multidrug resistance (TB-MDR) tuberculosis in Muna district in 2013–2015. *Scientific Journal of Public Health Students*. 2017;2(6):1–10. ISSN: 2502-731X.
23. Chaidir L, Sengstake S, de Beer J, et al. Mycobacterium tuberculosis genotypic drug resistance patterns and clustering in Jayapura, Papua, Indonesia. *The International Journal of Tuberculosis and Lung Disease*. 2015:428–433. https://doi.org/10.5588/ijtld.14.0350 (19 December 2014).
24. Pratama IGBA, Darwinata AE, Hendrayan ME. Characteristics of pulmonary tuberculosis patients with multidrug resistance in Sanglah Hospital, Bali in 2017–2018. *Jurnal Medika Udayana*. 2021;10(4):63–67. ISSN: 2597-8012.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Efficacy of TrueNat cartridge extracted DNA for detecting drug resistant tuberculosis by line probe assay

*Parul Jain, Rashmi Ratnam, Urmila Singh, Vijay Kumar, Amita Jain**

Department of Microbiology, King George's Medical University, Lucknow-226003

ARTICLE INFO

Article history:
Received 12 February 2022
Received in revised form
13 April 2023
Accepted 2 May 2023
Available online 5 May 2023

Keywords:
Colony forming units
Deoxyribonucleic acid
Diagnosis
Drug resistant tuberculosis
Temperature

ABSTRACT

Background: Currently for diagnosing *Mycobacterium tuberculosis* and its drug resistance, two sputum samples are required. One of them is subjected to TrueNat™ and if positive the other sample is subjected to line probe assay (LPA). This study was done to evaluate whether TrueNat extracted DNA can be directly used for performing LPA in a diagnostic laboratory setting to decrease patient turn-around time.

Methods: Total 45 smear positive sputum samples were subjected to TrueNat™ MTB detection and first and second line (FL and SL) LPA testing in parallel. DNA extracted by Trueprep® Cartridge was also tested by LPA and results were compared. Further, TrueNat extracted DNA from 20 samples was divided into 6 aliquots each, two of which were stored at 4 °C, 37 °C and 55 °C (under humidification) each. One aliquot from stored DNA at each temperature was used for FL & SL LPA on day three and the other on day eight. The blots thus obtained were compared with those of conventional LPA at day 1.

Results: For FL-LPA, TrueNat extracted DNA gave valid results for all 45 (100%) samples but conventionally extracted DNA could give results for 44 (97.8%) samples. Likewise, for SL-LPA, valid results were obtained for 40 (88.9%) and 35 (77.8%) samples respectively using TrueNat extracted DNA and conventionally extracted DNA respectively. All samples with invalid LPA results had Ct values $\geq$ 28 by TrueNat PCR. LPA results were obtained for all the 20 samples using stored DNA at all temperatures and duration.

Conclusions: TrueNat extracted DNA can be used for performing LPA under field conditions for selected samples.

1. Introduction

As per the WHO report, in 2019 it was estimated that about 10 million people suffered from tuberculosis (TB) worldwide among which 465,000 were Multi Drug Resistant/Rifampicin Resistant -TB (MDR/RR) new cases. India contributed to the highest burden of disease corresponding to 26% and 27% of the total TB cases and MDR/RR TB cases respectively.[1]

* Corresponding author. Department of Microbiology, King George's Medical University, Lucknow - 226 003. Tel.: +9415023928.
E-mail address: irluplno@rntcp.org (A. Jain).

https://doi.org/10.1016/j.ijtb.2023.05.002

Currently, the main diagnostic modalities used for tuberculosis and its drug resistance includes microscopy for acid-fast bacteria, automated liquid culture and drug susceptibility tests, rapid molecular tools such as GeneXpert (Cepheid, USA) and first and second line Line probe assay (LPA). India has recently adopted the indigenously developed and WHO endorsed TrueNat™ (Molbio Diagnostics, Goa, India) (will henceforth be referred to as TrueNat) as the point of care and upfront testing tool to diagnose TB and MDR TB.[2,3] The technology comes as a portable, battery-operated device that does not require sophisticated infrastructure, controlled temperature environment, consistent electrical supply or highly skilled manpower.

As per the current diagnostic algorithm developed by the National Tuberculosis Elimination Program (NTEP), India, the peripheral laboratories should collect an additional sputum sample for all patients testing positive for TB (regardless of their Rifampicin resistance status) by TrueNat/GeneXpert and send it to the linked intermediate reference laboratories (IRLs) for further work-up,[2] which includes line probe assay for resistance determination and if indicated liquid culture drug susceptibility testing (LC-DST) for drugs like pyrazinamide, moxifloxacin, linezolid, clofazimine, bedaquiline and delamanid.[2] Arrangement of additional sputum sample and its subsequent transportation using cold chain is prone to increase the patient turn around time, laboratory work and test cost. Additionally collected second sample also needs to be decontaminated, examined by smear microscopy for acid-fast bacilli and then subjected to DNA extraction before performing LPA. Besides bacteria in sputum may shed intermittently and second sample from previously positive patient may turn negative, which is not useable for LPA.

Uttar Pradesh (UP) is the largest state of India in terms of population and also has the highest burden of TB cases. Across the state, the NTEP has established about 460 TrueNat sites for TB diagnosis. Since DNA extraction of *Mycobacterium tuberculosis* is already being done at all the TrueNat sites, the authors speculated that if this DNA could be used directly for LPA, many of the aforesaid problems could be overcome. Therefore, this study was planned to study the efficacy of TrueNat cartridge extracted DNA in detection of genotypic drug resistance in *M. tuberculosis* by Line Probe Assay.

2. Material and methods

This pilot observational laboratory-experimental study was done at Tuberculosis laboratory, Department of Microbiology, King George's Medical University, Lucknow between September and October 2021. Ethical clearance was obtained from the Institutional Ethics committee (1354/Ethics/2021).

2.1. Efficacy of TrueNat extracted DNA

Of the sputum samples received at the laboratory, 70 consecutive good quality samples and which had sufficient quantity (>3 ml) were selected. These samples were decontaminated and subjected to fluorescent microscopy for acid-fast bacilli. Total 45 samples were positive which were then subjected to first (GenoType MTBDR *plus*) and second (GenoType MTBDR *sl ver 2.0*) line LPA (Hain Lifescience, Nehren, Germany) as per the manufacturer's instructions (on Day 1 of receipt of samples). Simultaneously, these samples were also subjected to DNA extraction by Trueprep® AUTO v2 Universal Cartridge based Sample Prep Device (Molbio Diagnostics, Goa, India) as per the manufacturer's instructions. Briefly, the provided lysis buffer was added to the sputum sample and the mixture thus obtained was loaded into the cartridge, which in turn was loaded on the device to enable further extraction and purification of the bacterial DNA. After 20 min of the process, extracted DNA elute was collected in an elute collection tube (ECT) for further processing. Each sample was repeatedly subjected to extraction so as to obtain the desired quantity of DNA.

The purified DNA obtained was subjected to Real Time PCR for detection of *M. tuberculosis* by TrueNat MTB-Rif Dx Kit using Truelab® Uno Dx Real Time Quantitative micro PCR Analyzer (Molbio Diagnostics, Goa, India) and the cycle threshold (Ct) values were recorded. The extracted DNA was also directly used for amplification for first and second line LPA (Fig. 1). For amplification step of LPA, 5 μl DNA template was added to 45 μl of amplification mix (containing 10μl of AM-A & 35μl of AM-B provided in the LPA kits) in a PCR tube and then the recommended cycling conditions were used. Hybridization of the amplified products was performed in GT BLOT- 48 machine (Hain Life Science GmbH, Nehren, Germany) according to manufacturer's instructions. The blots thus obtained were left to dry and results were interpreted as either *M. tuberculosis* resistant, sensitive or invalid for various anti-tubercular drugs.

The blots obtained by LPA from conventionally extracted DNA and from TrueNat extracted DNA was compared to know its efficacy.

2.2. Performance of TrueNat extracted DNA under field simulated conditions

Of the 45 Truenat extracted DNA samples, 20 samples with different drug resistance patterns, cycle threshold (Ct) values < 25 and with quantity >30μl were selected. DNA from each sample was fractionated into six aliquots of 5–6 μl each, of which a set of two aliquots were kept at different temperatures i.e. 4 °C, 37 °C and 55 °C (under humidification). One aliquot from each temperature was subjected to LPA after 3 days of incubation and the other after 8 days. The blots obtained from extracted DNA stored at different temperatures and durations were compared with the blots obtained on Day 1 using conventionally extracted DNA. The study design is shown in Fig. 1.

2.3. Statistical analysis

Significance of different Ct values was calculated by Mann Whitney U test using MedCalc online software (https://www.medcalc.org/calc/).

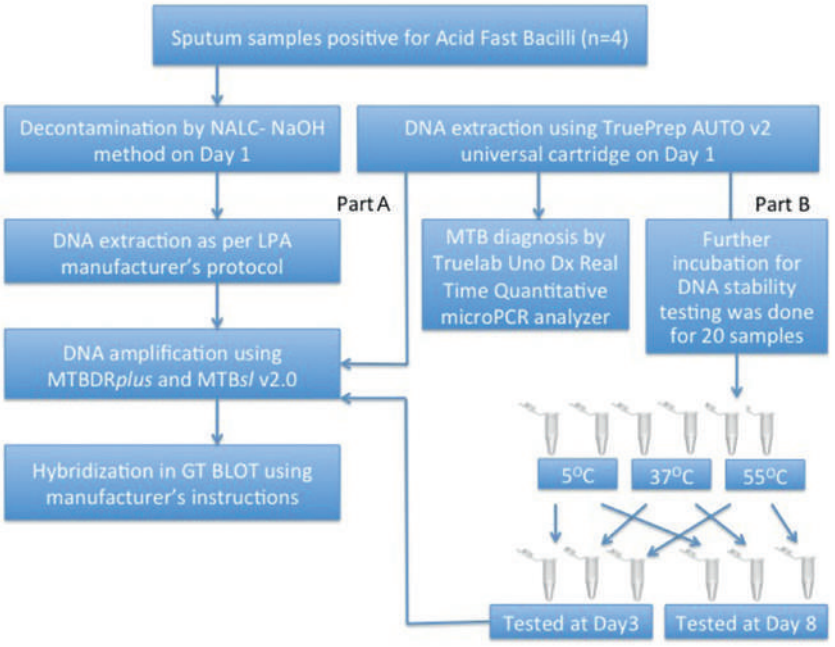


Fig. 1 – Flowchart showing the study design. Part A of the study consisted of comparing blots obtained using extracted DNA with Trueprep and conventionally extracted DNA. Part B consisted of stability testing of DNA at different temperatures and duration wherein blots obtained using stored DNA were compared with blots obtained on Day 1 by conventional LPA.

3. Results

3.1. *Patient details and TrueNat results*

Of the 45 cases testing positive by Truelab® Uno Dx Real Time Quantitative micro PCR analyzer, 34 were rifampicin resistant, six were rifampicin sensitive and five gave rifampicin indeterminate results. The mean age, gender distribution, median Ct values and the corresponding mycobacterial load in terms of colony forming units per milliliter of sample (CFU/ml), as given by the machine, for the rifampicin sensitive, resistant and indeterminate cases is mentioned in Table 1.

The Ct values of samples positive for *M. tuberculosis* by TrueNat ranged from 16.2 (corresponding to 3.8×10^7 CFU/ml) to 31.9 (corresponding to 4×10^2 CFU/ml). There was no significant difference in the Ct values (p value = 0.027, Z score = −1.10) or mycobacterial loads (p value = 0.42, Z score = 0.813) for *M. tuberculosis* between samples with rifampicin indeterminate and determinate (resistant or sensitive) results.

3.2. *Diagnostic accuracy*

When results of LPA obtained with DNA extracted by the two methods were compared, it was observed that for first line LPA an interpretable result was obtained for all the 45 (100%) samples using TrueNat extracted DNA but for 44 (97.8%) samples using conventionally extracted DNA. Likewise, for second line LPA interpretable results were obtained

Table 1 – Patient demographic and laboratory data (Ct: cycle threshold value, CFU/ml: colony forming unit per milliliter of sputum, LPA: Line probe assay).

Patient characteristics	Rifampicin Sensitive (n = 6)	Rifampicin Resistant (n = 34)	Rifampicin Indeterminate (n = 5)
Mean Age (Range)	40.8 years (22–71)	32.3 years (16–65)	32.6 years (20–63)
Male/Female	2/4	21/13	3/2
Median Ct values of Truenat Real Time PCR	28.4 (25.4–31.1)	23.8 (16.2–31.2)	26.7 (20–31.9)
CFU/ml	4.4×10^4 (1.8×10^3–$1.5 \times 10^{5)}$	3.3×10^6 ($1.2 \times 10^3 - 3.8 \times 10^7$)	5.8×10^5 ($4.0 \times 10^2 - 2.7 \times 10^6$)
Result obtained in First line LPA from sample	6 (100%)	34 (100%)	4 (80%)
Result obtained in First line LPA from TruePrep extracted DNA	6 (100%)	34 (100%)	5 (100%)
Result obtained in second line LPA from sample	2 (33.3%)	31 (91.2%)	2 (40%)
Result obtained in second line LPA from TruePrep extracted DNA	3 (50%)	32 (94.1%)	5 (100%)

with 40 (88.9%) and 35 (77.8%) samples using TrueNat extracted DNA and conventionally extracted DNA respectively (Table 1).

Indeterminate results for rifampicin, isoniazid, fluroquinolones and SLIDs were correlated with Ct values obtained with TrueNat PCR. Indeterminate results for first line drugs were obtained at Ct value more than 32 when LPA was done from conventionally extracted DNA. For second line drugs indeterminate results were obtained at Ct values ≥ 28. But even at such Ct values interpretable results were obtained for 5 samples with TrueNat extracted DNA (Table 2).

3.3. *Effect of temperature and duration on LPA results done from TrueNat extracted DNA*

20 samples were tested for the effect of temperature and duration as shown in Fig. 1. First and second line LPA results were obtained for all the 20 samples using stored DNA at all temperatures and duration and the results exactly matched those of LPA done on day 1.

4. Discussion

Following are the main findings of this study: 1) a good correlation of first and second line LPA results was observed using DNA obtained with TrueNat and that obtained conventionally; (2) for samples with Ct values more than 28, better results were obtained when TrueNat extracted DNA was used for LPA as compared to the conventionally extracted DNA; (3) there was no significant difference in the Ct values of samples giving determinate and indeterminate results for rifampicin; and (4) interpretable results for first and second line drugs can be obtained with LPA using the method described here, even if TrueNat gives a rifampicin indeterminate result.

In India, TrueNaat is a widely used technology for diagnosis and detection of rifampicin resistance of *M. tuberculosis*. For detection of *M. tuberculosis*, the TrueNat chip targets the ribonucleoside-diphosphate reductase gene (nrdB) that has a limit of detection of the order of 100 CFU/ml sputum sample.[4] If a sample tests positive, the extracted DNA is loaded on to another chip that detects mutations associated with rifampicin resistance by a probe melt analysis of the real time PCR products. It is often thought that a rifampicin indeterminate result is caused by paucibacillary mycobacterial load in the sample.[4] But in the present study rifampicin indeterminate results were obtained even with moderate mycobacterial loads (10^5-10^6 CFU/ml). The determinate results of rifampicin and isoniazid resistance and for the second line drugs were available for all these samples on LPA when TrueNat extracted DNA were used. This implies that certain other causes can also cause rifampicin indeterminate results and that even for such samples it is worth trying performing LPA using the TrueNat extracted DNA.

If results of this study are implemented in the program and the TrueNaat extracted DNA is transported to the labs for further testing, several advantages can be anticipated. 1) Patient will not have to visit the peripheral health center again to deposit another sputum sample for further testing. 2) DNA is not an infectious material posing biohazard danger and therefore transportation of DNA will be less expensive and more convenient than patients' sputum sample. Additionally, the amount of biohazard waste generated during sputum transportation in the form of sample packaging material can be reduced significantly. 3) Line probe assay is a multi-step process that includes both manual and semi-automated steps. Usage of extracted DNA will eliminate time-consuming manual steps and thus testing capacity of laboratories could be substantially increased without additional staff, machines and expensive kits/reagents. 4) It is often observed that of the *M. tuberculosis* positive samples sent to the reference labs, about 20% test negative for microscopy of the concentrated sputum sample by Auramine staining. Such samples need to be first cultured on liquid media and then indirect LPA is performed using the isolates obtained. The whole procedure takes about 15–45 days for diagnosis of drug resistance.[5] Using DNA samples that tested positive for *M. tuberculosis* directly for LPA is expected to drastically reduce turn around time for such samples. This technique will specially prove helpful in decreasing the time to treatment for MDR and pre-XDR cases.

TrueNaat extraction kit is supplied with a screw capped micro-centrifuge tube in which the final DNA elute is collected.[4] Looking at the advantages mentioned above, we propose that after the TrueNat testing for *M. tuberculosis* and rifampicin resistance is done and the sample tests positive for *M. tuberculosis* with a Ct value of less than 26 (10^4 CFU/ml), the left over DNA should be transported within that screw capped micro-centrifuge tube to the reference laboratories, as the diagnostic accuracy and stability of DNA at different temperatures and duration of transportation has already been shown. However, there will be certain challenges during its implementation. 1) The limitation with the present day

Table 2 – Correlation of Indeterminate results for first and second line LPA with mycobacterial loads.

Ct value	Mean DNA load in CFU/ml. (Range)	No. of samples giving Indeterminate results for FL drugs		No. of samples giving Indeterminate results for SL drugs	
		Using conventionally extracted DNA	Using TrueNat DNA	Using conventionally extracted DNA	Using TrueNat DNA
>15 to 20 (n = 6)	1.63×10^7 (2.7×10^6 to 3.8×10^7)	0	0	0	0
>20 to 25 (n = 18)	8.5×10^5 (6.2×10^4 to 2.2×10^6)	0	0	0	0
>25 to 30 (n = 14)	6.1×10^4 (2.3×10^3 to 1.8×10^5)	0	0	4	2
>30 to 35 (n = 7)	1.3×10^3 (4×10^2 to 2.3×10^3)	1	0	6	3

extraction kit is that the usual amount of elute obtained is 50μl and for running two PCRs for *M. tuberculosis* detection and rifampicin resistance estimation by TrueNat, at least 12μl DNA is required. This quantity may increase in case a test needs to be repeated. Transportation of the remaining small amount of DNA can be challenging and since at least 10μl of good quality DNA is required for one time testing by first line and second line–line probe assay, hence modifications are required in the extraction kit for obtaining a larger quantity of DNA. 2) Even if the results of the study are applied in the field, sputum sample transportation will still be required for samples with Ct values more than 28 (where both extracted DNA and sputum samples should be sent) and for samples where LC-DST needs to be performed. However, for majority of the samples turn around time for drug resistance testing will decrease thereby decreasing the patient turn around time. 3) The staff working at the peripheral and reference labs will need to be trained adequately regarding sample selection, packaging and transportation and for proper handing so as to avoid cross-contamination of the samples. Though, it is expected that if the technicians can be trained for conducting a molecular test in the field, they can easily be trained regarding these issues too.

A study has earlier been done on evaluation of mycobacterial genomic DNA from used Gene Xpert MTB/RIF cartridges for conducting first and second line LPA[6] but the authors obtained accurate results only with second line LPA for samples with at least medium (Ct value < 24) mycobacterial load. Effect of prolonged duration and ambient temperature was also not studied; hence its implementation in the field is still not convincible. Moreover, Gene Xpert machine is available only at reference labs in India and hence even if this technique works well, it may not be widely applicable in the Indian scenario.

Results of the present study may be considered by the policymakers to bring about appropriate changes in the NTEP regarding sample collection and transportation, which can be done in a phased manner. Limitation of this study was that phenotypic drug susceptibility testing or gene sequencing could not be conducted for first and second line drugs due to logistic reasons.

It can thus be concluded that DNA extracted with the TrueNaat technology can be safely used for direct performance of LPA. DNA remained stable even after 8 days of incubation at 37 °C and 55 °C under humidification, which reflect field conditions. This reflects that if DNA is transported in cold chain even during summers in India when the environmental temperature reaches up to 45 °C, valid results will be obtained.

Conflicts of interest

The authors have none to declare.

Acknowledgement

Authors thank the Central TB Division (New Delhi, India), State TB Cell, Lucknow and FIND, India for their financial support as well as kits and consumables support to our laboratory. Contribution of staff at our referral laboratory is greatly acknowledged.

REFERENCES

1. Executive summary, WHO Global Tuberculosis Report Executive summary 2020. Available from https://www.who.int/publications/i/item/9789240013131. Last accessed on 09.02.2022.
2. Guidelines on programmatic management of drug resistant tuberculosis in India-2019. Central TB Division, Ministry of Health and Family Welfare, Government of India. Available from https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4780&lid=3306 Accessed on 09.02.2022.
3. WHO consolidated guidelines on tuberculosis. *Module 3: diagnosis – rapid diagnostics for tuberculosis detection*. Geneva: World Health Organization; 2020. Available from https://www.who.int/publications/i/item/who-consolidated-guidelines-on-tuberculosis-module-3-diagnosis—-rapid-diagnostics-for-tuberculosis-detection.
4. Practical Guide to Implementation of Truenat™ Tests for the Detection of TB and Rifampicin Resistance. Stop TB partnership. Available from https://stoptb.org/assets/documents/resources/publications/sd/Truenat_Implementation_Guide.pdf. Accessed on 10.02.2022.
5. Global Laboratory Initiative- Mycobacteriology Laboratory Manual. https://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf; 2014.
6. Venter R, Minnies S, Derendinger B, et al. Extract from used Xpert MTB/RIF Ultra cartridges is useful for accurate second-line drug-resistant tuberculosis diagnosis with minimal rpoB-amplicon cross-contamination risk. *Sci Rep*. 2020;10:2633.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Original article

Adverse drug reactions in drug resistant pulmonary tuberculosis patients

*Shraddha Tewari**, *Rushi Patel*, *Karmay Shah*

Gujarat Cancer Society Medical College and Research Center, Ahmedabad, Gujarat, India

ARTICLE INFO

Article history:
Received 28 March 2023
Accepted 5 June 2023
Available online 7 June 2023

Keywords:
Adverse drug reactions
Multi drug resistance TB
Peripheral neuropathy

ABSTRACT

This study is conducted from year 2019–2022 in Gujarat Cancer Society medical college and research center, Ahmedabad. Out of total 275 patients on drug resistant TB regimen (all oral longer, shorter injectable and mono H) seen in opd, 55 patients presented with adverse drug reaction. Most commonly affected age group was 20–40 yr old. During the course of treatment 32.7% required hospitalization, of which 29% were admitted in ward, rest required ICU care. Maximum ADR occurred in first 30 days of starting ATT. Drug had to be withdrawn in 41.81% and in 32.7%, offending agent was withdrawn permanently. There was no mortality during the study.

1. Introduction

Tuberculosis is the leading infectious cause of death in the world (above HIV/AIDS). Incidence estimated around 10 million new cases per year worldwide. Approximately cause death 2 million per year.[1] In 2019, the 30 high TB burden countries accounted for 87% of new TB cases. 8 countries account for two thirds of the total, with India leading the count followed by Indonesia, China, Philippines, Pakistan, Nigeria, Bangladesh, and South Africa.[1] Ending the TB epidemic by 2030 is among the health targets of the United Nations Sustainable Developmental Goals (SDGs).[1] India has committed to END TB by 2025, five years ahead of the global SDG target. Prime Minister of India has launched TB free India campaign at Delhi END TB Summit on 13th march, 2018.[2] End the global TB Epidemic <10 case per 100,000. Recent global estimates indicate that about a half million new cases of rifampicin resistant TB (RR-TB) occurred in 2019 with 78% of them having confirmed MDR-TB.[2] Estimated number of MDR/RR-TB cases in India is 124,000 (9.1/lakh population)[2] A universal problem faced by clinicians is the poor patient compliance when adverse drug reactions (ADRs) occur. The main adverse effects of anti-TB drugs usually occur during the first 6–8 weeks of treatment the role of monitoring of the patient for early recognition of the ADRs has been underscored.[3] If the side-effects are not recognized on time and managed properly, they can lead to treatment interruption or can even be life threatening.

2. Materials and methods

This study was conducted on 55 patients who developed adverse drug reactions of MDR/XDR TB drugs in respiratory medicine opd or were hospitalized in DR TB ward at Gujarat Cancer Society Medical college& research center, Ahmedabad.

A detailed clinical history and complete (general and systemic) physical examination and necessary laboratory

* *Corresponding author*. O-303 shivabhi luxuria,makarpura main road,Vadodara,390013, Gujarat, India.
E-mail address: Tewari.shraddha23@gmail.com (S. Tewari).

https://doi.org/10.1016/j.ijtb.2023.06.001

investigation carried out and duly recorded. Patients were subjected to chest radiograph pa view, 2 sputum AFB smear examination, LFT, RFT, Suric acid, urine R/M, TSH, serum calcium, Serum magnesium, ECG and other routine hematological investigation. Special investigation were performed in relevant cases.

3. Inclusion criteria

- All patients on drug resistant regimen who present with ADRS associated with them.
- Both sexes and all age groups.

4. Exclusion criteria

- Patients who are on drug sensitive regimen.
- Patients who are not willing to participate in the study.

5. Result

ADRS were most common in age group 20 to 40 (61.82% of patients who presented with ADR were in the age group of 20–40.) (Diagram 1).

There is no sex preponderance in ADRs and incidence occurred with almost same frequency (Diagram 2).

Out of total 55 patients, 18 patients (32.7%) required hospitalization, of which 16 were admitted in ward and 2 in ICU (Diagram 3).

Most common adr reported by patients during this study was burning sensation in feet (linezolid induced). In patients who were on all oral longer regimen, most common ADR found incidentally by physicians on follow up visit was pink/black discolouration of skin due to clofazimine.

Most commonly affected system was skin (60%) followed by peripheral nervous system (29.09%), ENT (18.8%),GIT (16.36%) and hepatobiliary system (5.45%).(Diagram 4)

Only 1 patient was diagnosed with depression on all oral longer, patient tolerated regimen with antidepressants.

Patients on all oral longer reported burning and tingling sensation in their feet after almost 2 months therapy with linezolid. It was concluded to be drug induced neuropathy after HIV status and diabetes and vit b12 deficiency was ruled out and neurologist opinion was taken.

Out of 33 patients who had dermatological manifestations, 31 had pink discolouration due to clofazimine, one patient reported intense pruritus while on att and the other complained of rash. in both these patients withdrawal wasn't necessary, only supportive treatment was given 2 patients who were on MDR shorter regimen presented with joint pain and had hyperuricemia during 1st month. Patients were prescribed febuxostat and NSAIDS. Patient improved with supportive treatment alone and no withdrawal was necessary (Table 6).

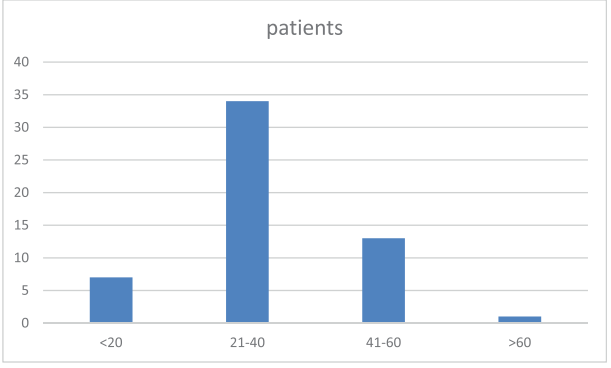


Diagram No 1 – ADR were most common in age group 20 to 40.

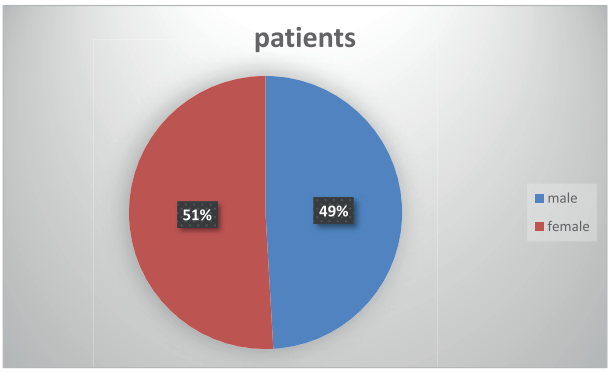


Diagram No 2 – There is no sex preponderance in ADRs and incidence occurred with almost same frequency.

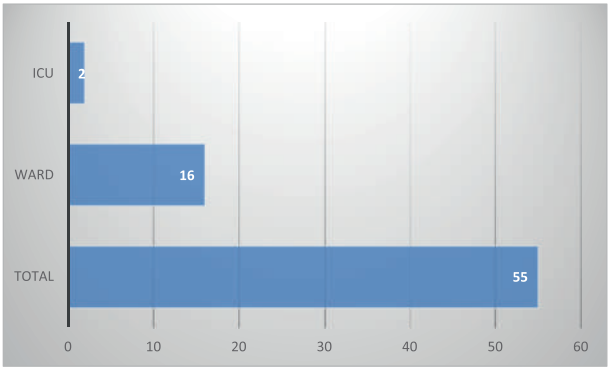


Diagram No 3 – Out of 18 hospitalized patients 16 were admitted in ward and 2 in ICU.

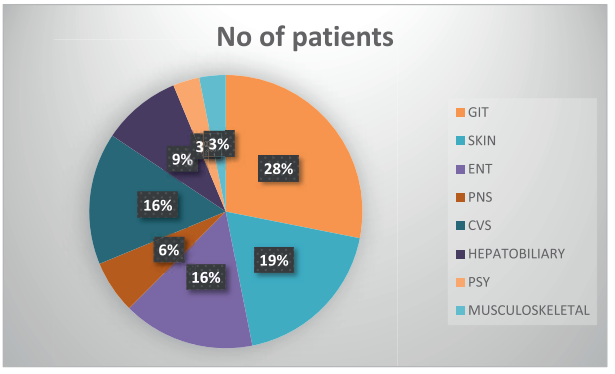


Diagram No 4 – Distribution of ADRs in first 30 days of starting DR TB.

Table 1 – Incidence of onset of ADRs from start of Anti TB drugs.

DAYS	NO OF PATIENTS	PERCENTAGE
0–30 days	33 PATIENTS	60%
31–60 days	14 PATIENTS	25.45%
61–90 days	7 PATIENTS	12.72%
>90 days	1 PATIENT	1.81%%
Total	55 PATIENTS	

Maximum ADRs occurred in first 30 days of starting MDR TB treatment.

Table 2 – Severity of ADRs according to severity assessment score. (MODIFIED HARTWIG AND SIEGEL SCALE)

SEVERITY	NO OF PATIENT (TOTAL = 55)	PERCENTAGE
MILD	16	29.09%
MODERATE	31	56.36%
SEVERE	8	14.54%

Table 3 – Incidence of lab finding in DR TB treatment adverse effect.

LAB FINDING	NO OF PATIENT (TOTAL = 55)	PERCENTAGE
Elevation of liver enzymes	3	5.45%
Hyperuricemia	2	3.63%
Hearing defect on audiometry	10	18.1%
Thrombocytopenia	1	1.81%
QTc prolongation	5	9.09%
Anaemia	1	1.81%

In 18% of patients who presented with ADRS, hearing loss was reported due to inj Kanamycin (shorter MDR regimen) and in all cases it was stopped and regimen was changed to all oral longer (Table 3).

1 patient presented with linezolid induced optic neuropathy which was henceforth stopped.

5 patients presented with QTc abnormalities while being on all oral longer,2 patients were of grade 2 and other two were of grade 3.4 of them were hospitalized and QTc prolonging drugs were put on hold and electrolytes corrected and then gradually reintroduced.

1 patient on modified all oral longer (bdq, Z, AM, CFZ, LZD, CS)presented with decreased urine output. patient was put on injectable thrice a week with regularly creatinine monitoring.

Maximum ADRS (60%)occurred in first 30 days of starting MDR TB treatment (Table 1).

In 14% of patients, ADR were severe (acc to modified hartwig and siegel scale) resulting in ICU requirement or some permanent harm to the patient (Table 2).

Majority of ADRs (56.36%) were of moderate severity resulting in withholding/discontinuation of the culprit drug along with the supportive treatment or requiring hospital admission or prolonging the duration of stay.

There was no mortality in the patients enrolled in my study.

In 23 patients (41.81%) drug had to be withdrawn, out of which in 18 patients it had to be withdrawn permanently (Table 4).

In 11 patients out of total 55, dose of the offending agent had to be reduced (linezolid in 10 patients and amikacin in 1 patient). (Table 5).

6. Discussion

Various studies have been conducted over time studying adverse effects of drug resistant tuberculosis. **"Profile of adverse reactions in drug resistant TB from Punjab" (Bharat Bhushan, Ramesh Chandra, NC Kajal).[4] Their study demonstrated that out of total 195 patients who reported ADR,** 63.58%, 18.46% and 17.94% were of mild, moderate and severe types respectively. The offending drug(s) had to be terminated in 12.08% of the patients in the study conducted in Punjab.

Table 4 – Incidence where drug was withdrawn due to ADR.

Regimen	Drug withdrawn	ADR	No of patient	Percentage	Reintroduced
Shorter	Kanamycin	SNHL[a]	10	18.18%	No
All oral longer	Linezolid	Peripheral neuropathy	7	12.72%	No
All oral longer	Bdq, FQ, Cfz	QTc prolongation	3	5.45%	Yes
Mono h	R and Z	ATT induced hepatitis	1	1.81%	Yes
Shorter	H, Z and Eto	ATT induced hepatitis	1	1.81%	Yes
All oral longer	Linezolid	Linezolid induced anaemia and thrombocytopenia	1	1.81%	No

[a] SNHL = sensorineural hearing loss.

Table 5 – Incidence when drug dose was reduced due to ADR.

Regimen	Drug dose reduced	ADR	No of patient	Percentage
All oral longer	Linezolid	Peripheral neuropathy	10	18.1%
Modified all oral longer	Amikacin	Acute Kidney Injury	1	1.82%

Table 6 – Incidence when regimen was not altered and supportive treatment was given.

System	No of patient	Supportive treatment
GIT	9	Antiemetics
SKIN	33	Sunscreen and antihistamines
PSY	1	Antidepressants
CVS	1	Electrolytes corrected and ECG monitored
MUSCULOSKELETAL	2	Febuxostat given with NSAIDS

In our study, 29.09% were of mild severity, 56.36% were of moderate severity and rest 14.55% were of severe nature warranting either ICU admission or were responsible for permanent harm to the patient e.g., linezolid induced peripheral neuropathy and kanamycin induced sensorineural hearing loss. In our study offending drug had to be terminated permanently in 32.7%, like linezolid induced myelosuppression and linezolid induced peripheral neuropathy and kanamycin induced sensorineural hearing loss.

In another study conducted in Lucknow, King George medical college in 2009, **Frequency of adverse events observed with second-line drugs among patients treated for multidrug-resistant tuberculosis (Rajendra Prasad, Abhijeet Singh, Rahul Srivastava, Giridhar b Hosmane, Ram Awadh Singh, Amita Jain)**[5] Ocut of total 98 patients, 46% patients reported ADR. Most common affected system was GIT in 40.3%, ototoxicity was seen in 23.6%, neurological in 17.6%. In 17.4% drug was permanently discontinued due to severe ADR.

In our study out of total 275 patients, ADR was seen in 20% patients. Most common ADR reported by patients was burning and tingling sensation in feet due to linezolid in 29.09%,. Second most common ADR seen was ototoxicity (18.8%), followed by GIT manifestations (16.36%).

In another study "Multidrug-Resistant Tuberculosis: A Systematic Review and Meta-analysis" conducted in china[6] (Shanshan Wu, Yuelun Zhang, Feng Sun, Mingting Chen, Lin Zhou, Ni Wang, Sivan Zhan) Of the 5346 patients included, 2602 (57.3%) experienced at least 1 kind of ADE. The 3 most common side effects were gastrointestinal disorders (32.1%), ototoxicity (14.6%), and psychiatric disorders (13.2%). Additionally, among 1519 patients who developed ADEs with available data of impact on MDR-TB therapy, 70.4% required change of MDR-TB treatment.

In another similar study in Pakistan, Occurrence of adverse events in patient receiving community-based therapy for multidrug-resistant tuberculosis in Pakistan(Arshad Javaid, Mazhar Ali Khan, F aheem Jan, Mifra Rauf, Mir Azam Khan, Anila Basit, Sumaira Mehreen)[7] Out of 200 cases, 155 (77.2%)presented with atleast 1 adverse drug event. psychiatric adverse events seen in 70%. In 13.5% one drug was discontinued temporarily.

In our study drugs which had to be removed temporarily were in 9.09%, ADR seen were QTc prolongation in patients who were on Bdq (all oral longer) and DR TB induced hepatitis in patients who were on shorter regimen.

7. Conclusion

All these studies depict that ADRs are commonly seen in DR TB regimens. Hence vigilance is required for proper screening of patients and regular follow up evaluation needs to be done to rule out ADR. Some ADRs are severe enough to warrant discontinuation of the drug either temporarily or permanently. Integrated approach by different specialities is required for proper management of ADRs. Pre-treatment evaluation is imperative before starting DR TB regimen as to screen patients who might be more at risk of developing ADRs.

There is a transition to shorter oral Bdq regimen from current injectable containing shorter regimen as many patients had to be shifted to different regimen due to ADR.

More studies need to be done to find drugs with lesser adverse effects which can be given for longer duration. Newer regimens are being studied which are shorter and safer for example BPAL regimen. Various trials are under process like BEAT TB trial and NIX TB trial.

Newer drugs such as pretomanid, sutezolid, SQ 109, AZD 5847, TBI -166 are being developed with improved efficacy and better safety profile.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. *WHO Global Tuberculosis Report*. 2021.
2. WHO. *Global tuberculosis report 2020 [internet]*; 2020. https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf. Accessed March 16, 2021.
3. Banu Eris Gulbay: Side effects due to primary anti tuberculosis drugs during the initial phase of therapy in 1149 hospitalised patients. Respiratory medicine 2016 (doi 10.1016/j.rmed.2006.01.014)
4. Bhushan B, Chander R, Kajal NC, Ranga V, Gupta A, Bharti H. Profile of adverse drug reactions in drug resistant tuberculosis from Punjab. *Indian J Tuberc*. 2014 Oct;61(4):318–324. PMID: 25675695.
5. Prasad Rajendra, Singh Abhijeet, Srivastava Rahul, Hosmane Giridhar b, Singh Ram Awadh, Jain Amita. Frequency of adverse events observed with second-line drugs among patients treated for multidrug-resistant tuberculosis. *Indian J Tuberculosis*. 2016. https://doi.org/10.1016/j.ijtb.2016.01.031.
6. Multidrug-Resistant Tuberculosis: A Systematic Review and Meta-Analysis" Conducted in china (Shanshan Wu, Yuelun Zhang, Feng Sun, Mingting Chen, Lin Zhou, Ni Wang, Sivan Zhan.
7. Javaid A, Khan MA, Jan F, Rauf M, Khan MA, Basit A, Mehreen S. Occurrence of adverse events in patient receiving community-based therapy for multidrug-resistant tuberculosis in Pakistan. *Tuberk Toraks*. 2018. https://doi.org/10.5578/tt.64054.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Original article

In-silico transcriptome analysis of antibiotic-treated *Mycobacterium tuberculosis* identifies novel antibiotic resistance factors

Shobana Sundar

Department of Biotechnology, PSG College of Technology, Coimbatore, India

ARTICLE INFO

Article history:
Received 23 June 2022
Accepted 20 June 2023
Available online 22 June 2023

Keywords:
Transcriptome
Antibiotic resistance
Mycobacterium tuberculosis

ABSTRACT

The emergence of drug resistant *Mycobacterium tuberculosis* strains increases the burden on the treatment of tuberculosis. In this study, through *in-silico* transcriptome analysis of drug-treated *M. tuberculosis* samples, novel drug targets for the treatment of drug resistance in tuberculosis were identified. Gene expression datasets of tuberculosis patients samples treated with different antibiotics (Isoniazid, Rifampicin, Pyrazinamide, Bedaquiline and Linezolid) were considered in this study. DESeq2 was used to identify the differentially regulated genes. Novel genes which were up-regulated during antibiotic treatment were identified which could be antibiotic resistance factors. Further, to understand the resistance mechanism of the novel genes, we performed gene ontology and gene network analysis for the differentially up-regulated genes. Thus, the *in-silico* transcriptome analysis paves way for a deeper understanding of the antibiotic resistance in *M. tuberculosis*.

1. Introduction

Tuberculosis (TB) is one of the leading causes of human death worldwide. Globally, there were around 5.8 million new cases reported for TB infection in 2020 and India is one of the worst affected countries [1]. Antibiotic resistance is a major impediment in the treatment of TB. The emergence of Multi-Drug Resistant (MDR) strains, which are resistant to at least the two most potent TB drugs, isoniazid and rifampin, and Extremely Drug Resistant (XDR) strains which are resistant to isoniazid, rifampin, a fluoroquinolone, and bedaquiline or linezolid. In this study, antibiotic resistance factors have been determined for samples treated with Isoniazid, Rifampicin, pyrazinamide, Bedaquiline and Linezolid.

Isoniazid (INH) is a front-line antibiotic used for the treatment of active and latent TB. It acts as a prodrug to inhibit mycobacterial cell wall formation. INH requires the activity of *katG*, a catalase-peroxidase that catalyses the formation of isonicotinic acyl radical, which immediately couples with NADH to form the nicotinoyl-NAD adduct. The nicotinoyl-NAD adduct tightly binds and blocks the activity of an enoyl reductase, *inhA* thereby inhibiting the biosynthesis of mycolic acids, which are the major components of the mycobacterial cell wall [2]. Resistance to INH commonly occurs due to mutations in the *katG* or *inhA* genes[3,4].

Abbreviations: TB, Tuberculosis; Mtb, *Mycobacterium tuberculosis*.

E-mail address: sbs.bio@psgtech.ac.in.

https://doi.org/10.1016/j.ijtb.2023.06.010

Rifampicin (Rifampin) is an antibiotic usually given for the treatment of latent tuberculosis and is always used in combination with other drugs such as pyrazinamide, isoniazid, and ethambutol. The mechanism of action of rifampicin is by inhibiting the bacterial DNA-dependent RNA synthesis by blocking the bacterial DNA-dependent RNA polymerase [5]. Resistance o rifampicin is usually attributed to mutations in the *rpoB* gene which encodes the beta subunit of RNA polymerase [6].

Pyrazinamide is only used in combination with isoniazid and rifampicin for the treatment of TB. Pyrazinamide is a prodrug, where the enzyme pyrazinamidase converts pyrazinamide to the active form pyrazinoic acid. Its mechanism of action was hypothesized that pyrazinoic acid blocks the synthesis of coenzyme A. By forming a weak interaction with aspartate decarboxylase (PanD), pyrazinoic acid causes the enzyme to degrade [7]. *pncA* mutations which encode a pyrazinamidase and convert pyrazinamide to pyrazinoic acid contributes resistance to Pyrazinamide [8].

Bedaquiline is normally used to treat active TB and MDR-TB patients and it is a component of the experimental BPaMZ combination treatment (https://www.tballiance.org/portfolio/regimen/bpamz). The mechanism of action of Bedaquiline acts by blocking the ATP synthase proton pump, as ATP production is required for cellular energy production of the mycobacteria to survive [9] Common known mechanisms of Bedaquiline resistance include mutations in the genes *atpE*, Rv0678 [10].

Linezolid is a bacterial protein synthesis inhibitor as it prevents the formation of the initiation complex and is used to treat MDR patients [11]. The intrinsic resistance to linezolid was mainly developed due to the activity of efflux pumps which can pump out linezolid easily out of the cell. Mutations in the *rrl* and *rplC* genes are the main causes of linezolid resistance in Mtb [12,13].

In this study, *in-silico* transcriptome analysis of Mtb samples treated with Isoniazid, Rifampicin, Pyrazinamide, Bedaquiline and Linezolid has been used to identify novel antibiotic resistance factors.

2. Methods

2.1. Data retrieval

The data used for this analysis were retrieved from the NCBI SRA database [14]. Transcriptome profiling of *Mycobacterium tuberculosis* H37Rv to primary drugs such as Isoniazid (SRA Accession: SRP303368), Rifampicin (SRA Accession: SRP305868), Pyrazinoic acid (SRA Accession: SRP306121), Bedaquiline (SRA Accession: SRP305672) and Linezolid (SRA Accession: SRP304915) were analyzed in this study as resistance for these drugs have been reported earlier. All these accessions were run on Illumina NextSeq 500 platform. Except for SRP306121, all other accessions have 27 samples, out of which 18 samples are treated with the drug, and the remaining 9 samples are left untreated. SRP306121 study has 18 samples, out of which 9 samples are treated and the other 9 samples are untreated.

2.2. Quality checking and assembly of raw reads

The tools available at the Galaxy Europe server [https://usegalaxy.eu/] were used for the analysis. The paired-end raw data in FASTQ format for each sample reported were individually analyzed for each of the primary drugs. To check for the presence of adapters and artifacts, the quality of the reads was assessed using FastQC [15]. Further, the high-quality FASTQ sequences were then aligned to the reference Mtb genome obtained from NCBI using HISAT2 [16]. HISAT2 is a sensitive aligner for the mapping reads with the reference genome. The aligned sequences were then assembled and assigned to genes using StringTie [17] using the Mtb reference genome annotation obtained from NCBI. StringTie measures the expression of each gene during assembly. To avoid partial read coverage and to ensure consistency of transcripts across all samples, the assemblies are merged using StringTie merge. The expression levels of all the transcripts are re-calculated using StringTie from the merged assembly.

2.3. Differential gene expression analysis

The count data was fed as input to DESeq2 [18] and was used to identify the differentially expressed genes between the control and drug-treated samples. For the identification of DEGs, the p-value cut-off was set to less than 0.05. Up-regulated genes were defined as those with a log2 Fold Change (log2 FC) more than 2 and down-regulated genes as those with a log2 FC less than −2. The list of up-regulated genes can be potential drug-resistant genes that seem to be overexpressed under drug treatment. PANTHER [19] was used to study the different gene ontologies of the up-regulated genes and the functional annotation pie chart has been retrieved.

2.4. Gene network analysis

STRING version 11.5 [20] was used for the analysis of protein–protein interactions of the up-regulated genes during antibiotic treatment. It is a database of known and predicted protein–protein interactions usually retrieved from Genomic Context Predictions, High-throughput Lab Experiments, Co-Expression, Automated Textmining and Previous Knowledge from other databases. StringApp of Cytoscape software has been used to determine protein–protein interactions of the up-regulated genes retrieved during antibiotic treatment.

3. Results and discussion

3.1. Differential gene expression analysis

3.1.1. Analysis of DEGs corresponding to isoniazid

The samples (SRA Accession: SRP303368) treated with Isoniazid were analysed for identification of DEGs, 71 genes were found to be up-regulated and 48 genes were down-regulated. The p-value and the log2 FC values for the up-regulated and down-regulated genes during isoniazid treatment are provided in Supplementary File S1. The Gene ontology analysis was performed for the up-regulated genes and is depicted in

Fig. 1. The majority of the genes identified were categorized as having a catalytic activity (GO: 0003824) and they are involved in metabolite interconversion (PC00262).

Genes with a log2 FC larger than 4 and a p-value less than 0.05 were chosen for further discussion. For samples treated with Isoniazid, genes iniA (Rv0342), iniB (Rv0341) and Rv1057 were found to be up-regulated.[21] demonstrated that the iniA gene (Rv0342) contributes to isoniazid drug resistance by activating an efflux pump that confers drug tolerance. They have further added that the iniA gene is strongly induced along with iniB and iniC (Rv0341 and Rv0343) by treatment of Mtb with Isoniazid. Rv1057 is a β-propeller gene of Mtb and is a regulatory target of the TrcRS two-component system.[22] have listed Rv1057 as one of the up-regulated Mtb genes upon addition of cysteine to INH-treated Mtb cultures. Therefore, iniA, iniB and Rv1057 are potential drug targets for the treatment of isoniazid drug resistance.

3.1.2. *Analysis of DEGs corresponding to rifampicin*

The samples (SRA Accession: SRP305868) treated with Rifampicin were analysed for identification of DEGs, 41 genes were found to be up-regulated and 44 genes were down-regulated. The p-value and the log2 FC values for the up-regulated and down-regulated genes during rifampicin treatment are provided in Supplementary File S1. The Gene ontology analysis was performed for the up-regulated genes and is depicted in Fig. 2. The majority of the genes identified were categorized as having a catalytic activity (GO: 0003824) and they are involved in metabolite interconversion (PC00262) and amino acid biosynthesis pathways.

Genes with a log2 FC larger than 3.5 and a p-value less than 0.05 were chosen for further discussion. For samples treated with Rifampicin, Rv0196 (Uncharacterized HTH-type transcriptional regulator) and Rv1471 (thioredoxin trxB1) were found to be up-regulated. Rv0196 is an uncharacterized HTH-type transcriptional regulator and studies relating Rv0196 to rifampicin resistance are still elusive.[23] have earlier reported an up-regulation of Rv1471 during the early transcriptional response in resuscitating Mtbtreated with rifampicin.

3.1.3. *Analysis of DEGs corresponding to pyrazinoic acid*

The samples (SRA Accession: SRP306121) treated with Pyrazinoic acid were analysed for identification of DEGs, 38 genes were found to be up-regulated and 2 genes were down-regulated. The p-value and the log2 FC values for the up-regulated and down-regulated genes during Pyrazinoic acid treatment are provided in Supplementary File S1. The Gene ontology analysis was performed for the up-regulated genes and is depicted in Fig. 3. The majority of the genes identified were categorized as having a catalytic activity (GO: 0003824) and they are involved in metabolite interconversion (PC00262) and in amino acid biosynthesis pathways.

Genes with a log2 FC larger than 4 and a p-value less than 0.05 were chosen for further discussion. For samples treated with Pyrazinoic acid, genes Rv0186A (mymT) and Rv2428 (ahpC) were found to be up-regulated. Rv0186A (mymT) is metallothionein that binds a minimum of four to six Cu + ions and it usually protects Mtb from copper toxicity. Rv2428

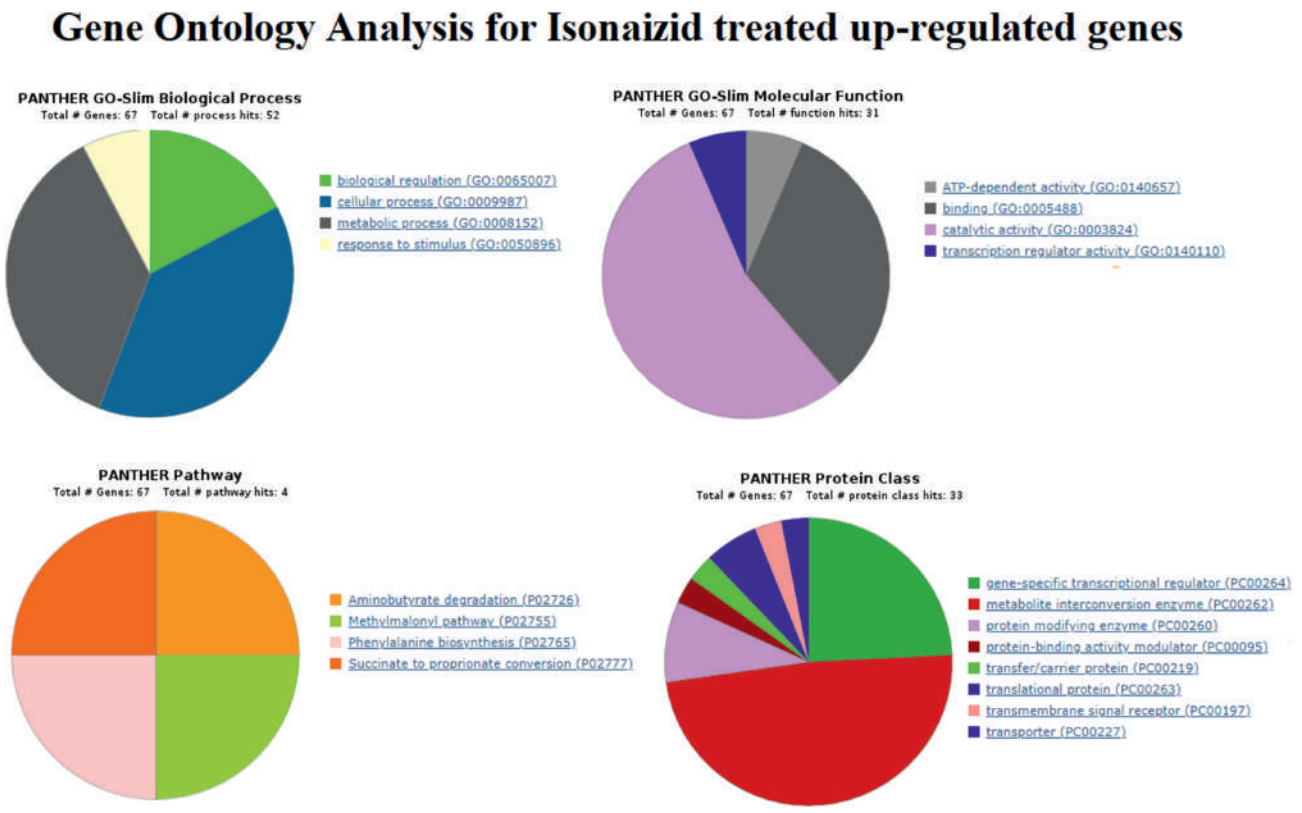


Fig. 1 – Title: Gene Ontology Analysis for Isoniazid treated up-regulated genes.

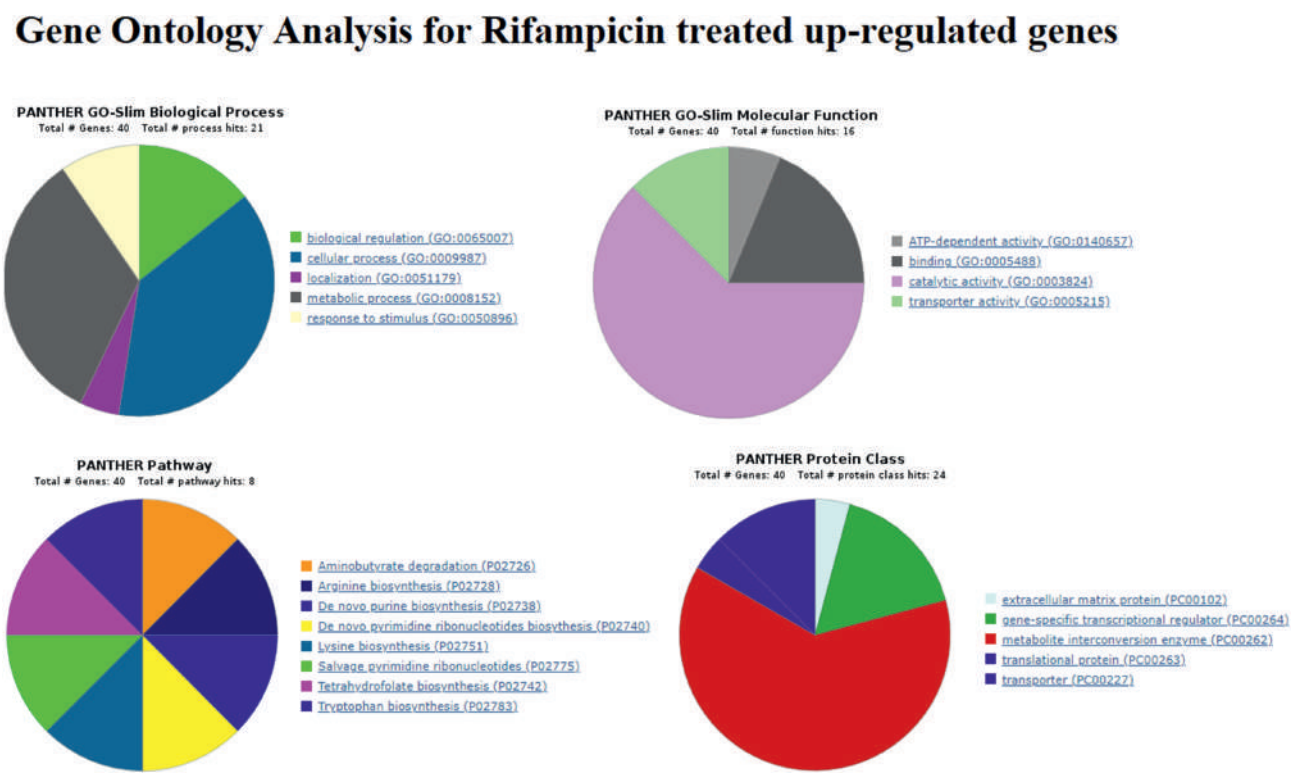


Fig. 2 – Title: Gene Ontology Analysis for Rifampicin treated up-regulated genes.

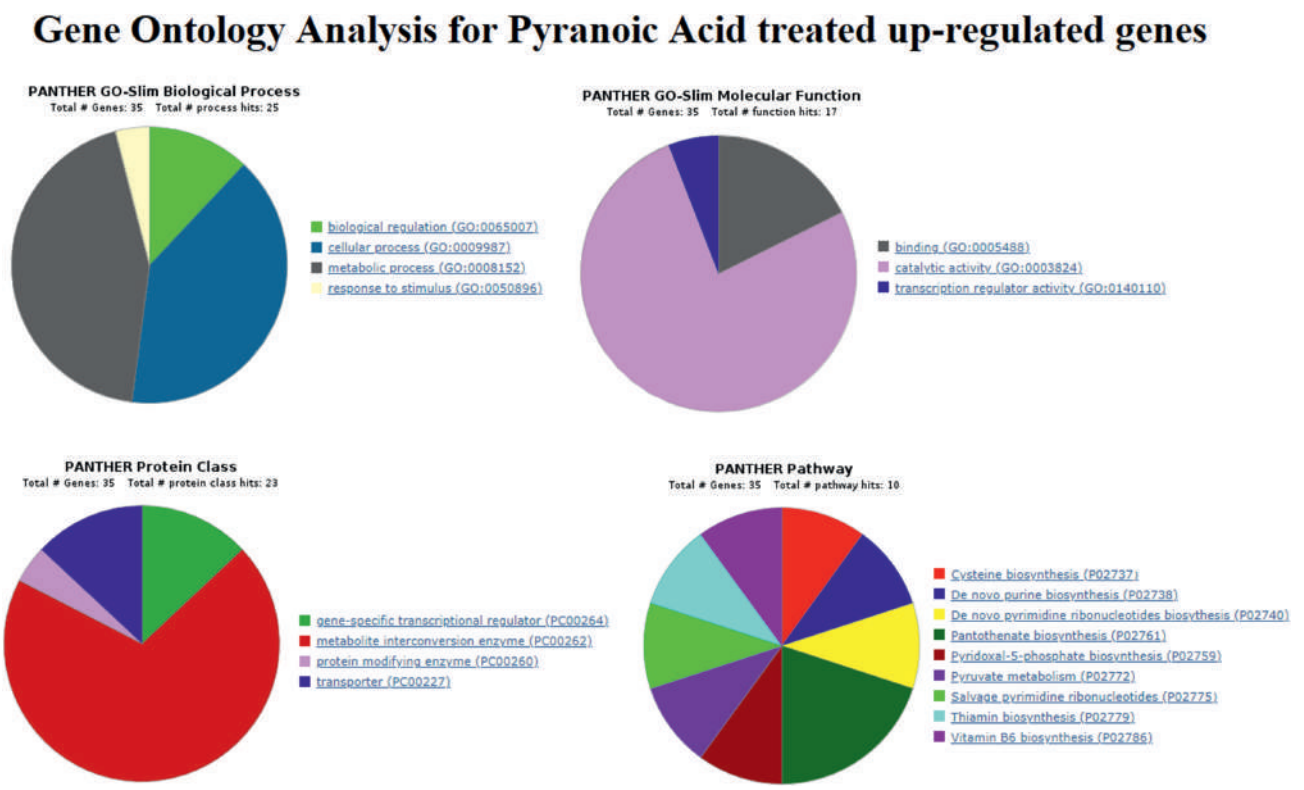


Fig. 3 – Title: Gene Ontology Analysis for Pyrazinoic acid treated up-regulated genes.

(ahpC) is a thiol-specific peroxidase that catalyzes the reduction of hydrogen peroxide and it protects the cell against oxidative stress [24]. Studies reporting on genes Rv0186A and Rv2428 for Pyrazinoic acid resistance are very vague.

3.1.4. *Analysis of DEGs corresponding to bedaquiline*

The samples (SRA Accession: SRP305672) treated with Bedaquiline were analysed for identification of DEGs, 13 genes were found to be up-regulated and 184 genes were down-regulated.

The p-value and the log2 FC values for the up-regulated and down-regulated genes during Bedaquiline treatment are provided in Supplementary File S1. The Gene ontology analysis was performed for the up-regulated genes and is depicted in Fig. 4. The majority of the genes identified were involved in binding (GO: 0005488) and they are involved in gene-specific transcriptional regulator (PC00264) and metabolite interconversion (PC00262).

Genes with a log2 FC larger than 2.3 and a p-value less than 0.05 were chosen for further discussion. For samples treated with Bedaquiline, genes Rv3306c (amiB1), Rv1571 and Rv0550c were found to be up-regulated. Rv3306c (amiB1) was predicted to have a para-aminobenzoyl-glutamate hydrolase activity and play an important role in the folic acid catabolic process, Rv1571 is an uncharacterized protein and Rv0550c is predicted to be an antitoxin vapB3. Studies on these genes reporting for bedaquiline resistance are very limited.

3.1.5. *Analysis of DEGs corresponding to linezolid*

The samples (SRA Accession: SRP304915) treated with Linezolid were analysed for identification of DEGs, 232 genes were found to be up-regulated and 133 genes were down-regulated. The p-value and the log2 FC values for the up-regulated and down-regulated genes during Linezolid treatment are provided in Supplementary File S1. The Gene ontology analysis was performed for the up-regulated genes and is depicted in Fig. 5. The majority of the genes identified were involved in cellular (GO: 0009987) and metabolic processes (GO: 0008152) and involved in catalytic activity (GO: 0003824). Most of them are involved in metabolite interconversion enzyme (PC00262).

Genes with a log2 FC larger than 5 and a p-value less than 0.05 were chosen for further discussion. For samples treated with Linezolid, genes Rv3197A (whiB7), Rv1645c (MPAB_Lcp_-cat domain-containing protein), Rv0654 (Carotenoid cleavage oxygenase), Rv1318c (Uncharacterized protein) and Rv2878c (Soluble secreted antigen MPT53) was found to be up-regulated. Rv3197A (whiB7) is a transcriptional regulator and contributes to intrinsic resistance by activating its expression and it regulates many genes such as Rv1258c, Rv1988, Rv2301, Rv2416c, Rv2725c for overproduction [25,26]. The apo-form is shown to have protein disulfide reductase activity [27] and is predicted to bind DNA. Rv1645c was predicted to contain the catalytic domain mostly found in the endoplasmic reticulum bound oxygenases mpaB' (MPAB2) and mpaB (MPAB) and in the rubber oxygenase (Lcp) that comprises highly conserved arginine and histidine residues. Rv0654 encodes for a putative carotenoid oxygenase and is conserved in other mycobacteria [28]. Rv2878c (MPT53) is a disulphide oxidoreductase that catalyzes the oxidation of reduced proteins [29]. Studies on these genes reporting for linezolid resistance are very scarce.

3.2. *Gene network analysis*

The up-regulated genes of the antibiotics treated were subjected to identify protein–protein interactions using string-APP in Cytoscape software. The protein–protein interactions networks for the up-regulated genes for the antibiotics treated are depicted in Fig. 6. The protein–protein interactions of the up-regulated genes under isoniazid treated mostly belong to fatty acid biosynthesis and metabolism (Supplementary file

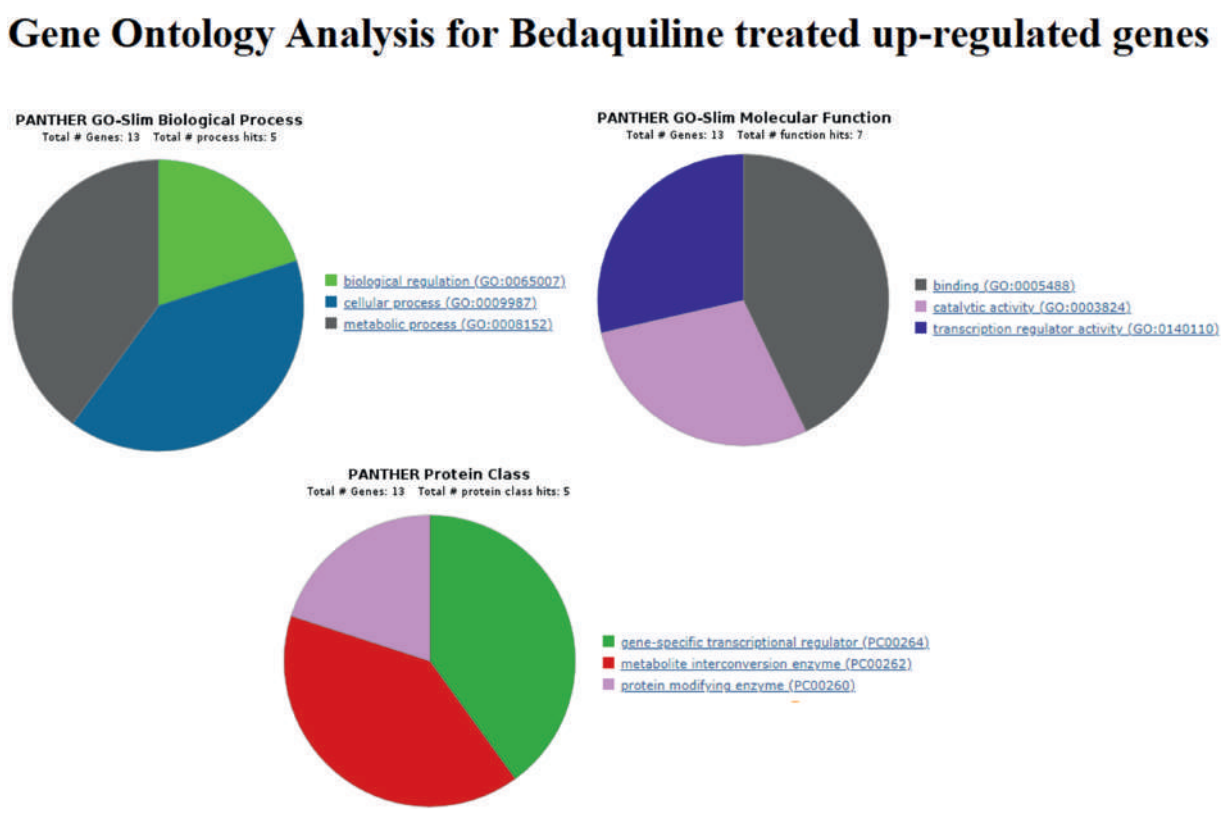


Fig. 4 – Title: Gene Ontology Analysis for Bedaquiline treated up-regulated genes.

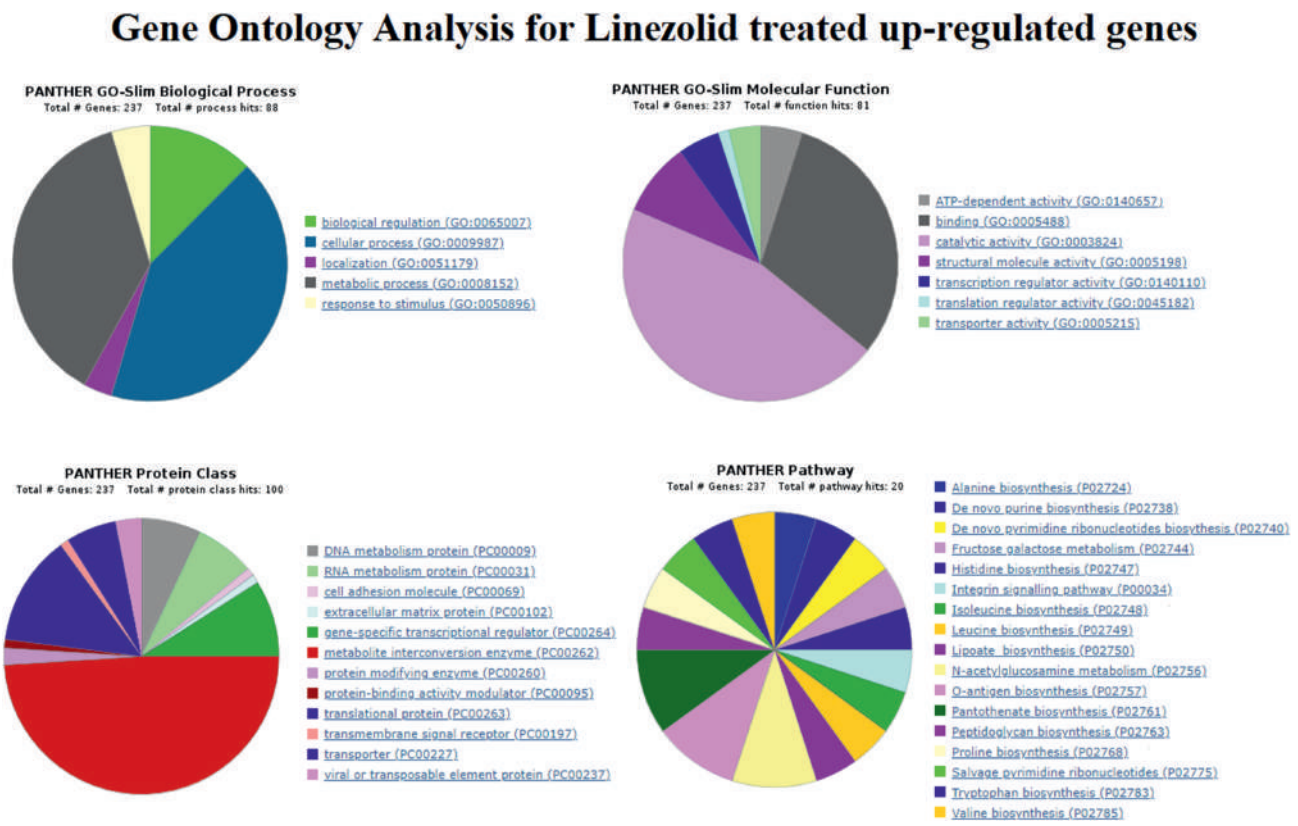


Fig. 5 – Title: Gene Ontology Analysis for Linezolid treated up-regulated genes.

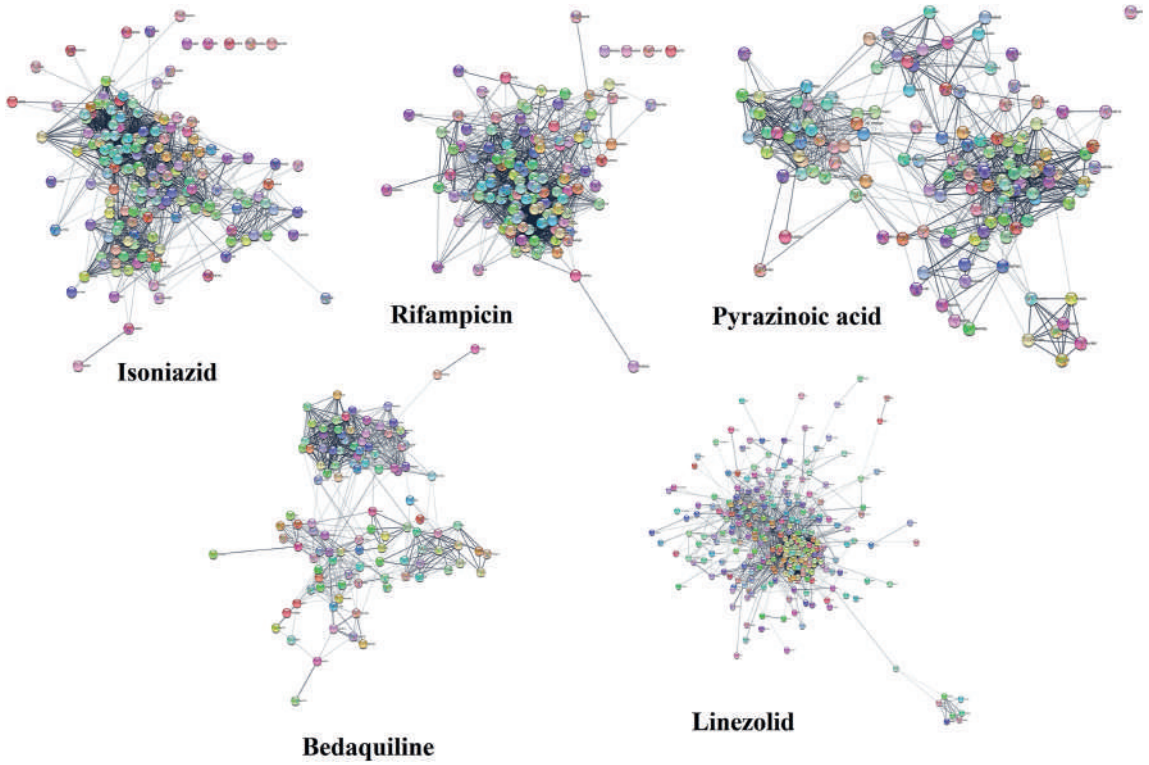


Fig. 6 – Title: Protein–protein interactions of the up-regulated genes during antibiotic treatment.

S2), the interactions network of the up-regulated genes for rifampicin treated mostly involved in binding function (Supplementary file S2), the interactions network of the up-regulated genes for pyrazinoic acid treated mostly involved in nucleotide biosynthesis and metabolism (Supplementary file S2), the interactions network of the up-regulated genes for Bedaquiline treated mostly involved in peptidoglycan biosynthetic process (Supplementary file S2) and the interactions network of the up-regulated genes for linezolid treated mostly involved in translational and protein export ((Supplementary file S2).

4. Conclusion

In this study in-silico transcriptome analysis of Mtb strains treated with antibiotics such as isoniazid, rifampicin, pyrazinoic acid, Bedaquiline and linezolid has been analysed to detect novel antibiotic resistance factors. Datasets have been retrieved from the NCBI SRA database. Gene expression analyses were performed using the DESeq2 tool for Mtb samples treated with antibiotics. Differentially regulated genes were identified and gene ontology analyses were performed for the up-regulated genes. Gene network analyses for the up-regulated genes were also performed to defer the antibiotic resistance mechanism. Thus, the in-silico analysis provides novel genes which could be antibiotic resistance factors that need further evaluation using experimental techniques.

Conflicts of interest

The authors has none to declare

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2023.06.010.

REFERENCES

1. *Global WHO Report on Tuberculosis*. 2021.
2. Suarez J, Ranguelova K, Jarzecki AA, et al. An oxyferrous heme/protein-based radical intermediate is catalytically competent in the catalase reaction of Mycobacterium tuberculosis catalase-peroxidase (KatG). *J Biol Chem*. 2009 Mar 13;284(11):7017–7029.
3. Bollela VR, Namburete EI, Feliciano CS, Macheque D, Harrison LH, Caminero JA. Detection of katG and inhA mutations to guide isoniazid and ethionamide use for drug-resistant tuberculosis. *Int J Tubercul Lung Dis*. 2016 Aug 1;20(8):1099–1104.
4. Bollela VR, Namburete EI, Feliciano CS, Macheque D, Harrison LH, Caminero JA. Detection of katG and inhA mutations to guide isoniazid and ethionamide use for drug-resistant tuberculosis. *Int J Tubercul Lung Dis*. 2016 Aug 1;20(8):1099–1104.
5. Calvori C, Frontali L, Leoni L, Tecce G. Effect of rifampicin on protein synthesis. *Nature*. 1965 Jul;207(4995):417–418.
6. Goldstein BP. Resistance to rifampicin: a review. *J Antibiot*. 2014 Sep;67(9):625–630.
7. Gopal P, Sarathy JP, Yee M, et al. Pyrazinamide triggers degradation of its target aspartate decarboxylase. *Nat Commun*. 2020 Apr 3;11(1):1, 0.
8. Scorpio A, Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nat Med*. 1996 Jun;2(6):662–667.
9. Worley MV, Estrada SJ. Bedaquiline: a novel antitubercular agent for the treatment of multidrug-resistant tuberculosis. *Pharmacotherapy*. 2014 Nov;34(11):1187–1197.
10. Singh BK, Soneja M, Sharma R, et al. Mutation in atpE and Rv0678 genes associated with bedaquline resistance among drug-resistant tuberculosis patients: a pilot study from a high-burden setting in Northern India. *International Journal of Mycobacteriology*. 2020 Apr 1;9(2):212.
11. Rodrıguez JC, Ruiz M, Lopez M, Royo G. In vitro activity of moxifloxacin, levofloxacin, gatifloxacin and linezolid against Mycobacterium tuberculosis. *Int J Antimicrob Agents*. 2002 Dec 1;20(6):464–467.
12. Wasserman S, Louw G, Ramangoaela L, et al. Linezolid resistance in patients with drug-resistant TB and treatment failure in South Africa. *J Antimicrob Chemother*. 2019 Aug 1;74(8):2377–2384.
13. Nambiar R, Tornheim JA, Diricks M, et al. Linezolid resistance in Mycobacterium tuberculosis isolates at a tertiary care centre in Mumbai, India. *Indian J Med Res*. 2021 Jul;154(1):85.
14. Srinivas V, Ruiz RA, Pan M, Immanuel SR, Peterson EJ, Baliga NS. Transcriptome signature of cell viability predicts drug response and drug interaction in. *Mycobacterium tuberculosis. Cell reports methods*. 2021 Dec 20;1(8), 100123.
15. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data.
16. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods*. 2015 Apr;12(4):357–360.
17. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol*. 2015 Mar;33(3):290–295.
18. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014 Dec;15(12):1–21.
19. Mi H, Ebert D, Muruganujan A, et al. PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. *Nucleic Acids Res*. 2021 Jan 8;49(D1):D394–D403.
20. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021 Jan 8;49(D1):D605–D612.
21. Colangeli R, Helb D, Sridharan S, et al. The Mycobacterium tuberculosis iniA gene is essential for activity of an efflux pump that confers drug tolerance to both isoniazid and ethambutol. *Mol Microbiol*. 2005 Mar;55(6):1829–1840.
22. Vilchèze C, Hartman T, Weinrick B, et al. Enhanced respiration prevents drug tolerance and drug resistance in Mycobacterium tuberculosis. *Proc Natl Acad Sci USA*. 2017 Apr 25;114(17):4495–4500.
23. Salina EG, Grigorov AS, Bychenko OS, et al. Resuscitation of dormant "non-culturable" Mycobacterium tuberculosis is characterized by immediate transcriptional burst. *Front Cell Infect Microbiol*. 2019:272.
24. Hillas PJ, del Alba FS, Oyarzabal J, Wilks A, de Montellano PR. The AhpC and AhpD antioxidant defense system of

Mycobacterium tuberculosis. *J Biol Chem*. 2000 Jun 23;275(25):18801–18809.
25. Morris RP, Nguyen L, Gatfield J, et al. Ancestral antibiotic resistance in Mycobacterium tuberculosis. *Proc Natl Acad Sci USA*. 2005 Aug 23;102(34):12200–12205.
26. Burian J, Ramón-García S, Sweet G, Gómez-Velasco A, Av-Gay Y, Thompson CJ. The mycobacterial transcriptional regulator whiB7 gene links redox homeostasis and intrinsic antibiotic resistance. *J Biol Chem*. 2012 Jan 2;287(1):299–310.
27. Alam MS, Garg SK, Agrawal P. Studies on structural and functional divergence among seven WhiB proteins of Mycobacterium tuberculosis H37Rv. *FEBS J*. 2009 Jan;276(1):76–93.
28. Scherzinger D, Scheffer E, Bär C, Ernst H, Al-Babili S. The Mycobacterium tuberculosis ORF Rv0654 encodes a carotenoid oxygenase mediating central and excentric cleavage of conventional and aromatic carotenoids. *FEBS J*. 2010 Nov;277(22):4662–4673.
29. Goulding CW, Apostol MI, Gleiter S, et al. Gram-positive DsbE proteins function differently from gram-negative DsbE homologs: a structure to function analysis of DsbE from Mycobacterium tuberculosis. *J Biol Chem*. 2004 Jan 30;279(5):3516–3524.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Original article

Pattern and characteristics of mutations conferring resistance to second line drugs in *Mycobacterium tuberculosis* isolates of pulmonary and extrapulmonary TB samples

Ramakant Dixit [a,*], *Emil Mohan* [a], *Ankur Gupta* [a], *Tarun Patni* [b]

[a] *Department of Respiratory Medicine, J L N Medical College, Ajmer, India*
[b] *State TB Demonstration and Training Centre, Government of Rajasthan, Ajmer, India*

ARTICLE INFO

Article history:
Received 24 February 2023
Received in revised form
26 June 2023
Accepted 4 July 2023
Available online 8 July 2023

Keywords:
Mutation
Fluoroquinolones
Aminoglycosides
Tuberculosis
Drug resistance

ABSTRACT

Background & objectives: The purpose of present study is to analyse the distribution and pattern of genetic mutations in PRE-XDR-TB and extensive drug resistant *Mycobacterium tuberculosis* (XDR-TB) using second-line line probe assay and to compare them with different parameters.

Method: Sputum, Lymph node aspirate and cold accesses from patients with rifampicin resistant Tuberculosis were subjected to first line and second line Probe Assay (Genotype MTBDRsl by Hain Life Science, Germany) to assess additional drug resistance to fluroquinolones (Levofloxacin & Moxifloxacin) and Aminoglycosides (Amikacin, Ofloxacin and Kanamycin). The genetic mutation pattern was analysed and compared with demographic, clinical and other parameters.

Results: The final study population included 123 fluoroquinolone resistant isolates including 14 isolates with additional second line aminoglycosides drug resistance. The most frequent mutation observed among Gyr A drug resistance mutation was D94G (Gyr A MUT3C, 50/123,40%) corresponding to high level resistance to levofloxacin and moxifloxacin. The most frequent wild type mutant among Gyr A gene locus was WT 3 (85/123,69%). The most common mutation among second line aminoglycoside resistant isolates was at eis WT2 (7/14,50%) followed by rrs MUT 2 (4/14,29%).

Conclusions: GyrA MUT3C (Asp94Gly) was the most common mutation in Gyr A gene locus in *M. tuberculosis* causing high level levofloxacin and moxifloxacin resistance. Patients with Asp94Gly mutation was significantly associated with underweight body mass index ($p = 0.026$). This study also observed that history of anti-tuberculosis therapy is a risk factor for FQ drug resistance mutations ($p < 0.001$).

* *Corresponding author.*
E-mail address: dr.ramakantdixit@gmail.com (R. Dixit).

https://doi.org/10.1016/j.ijtb.2023.07.003

1. Introduction

Tuberculosis (TB) is a communicable disease with significant morbidity and mortality worldwide. Before COVID-19 pandemic, TB was among leading causes of death from an infectious agent, ranking above HIV/AIDS.[1] Ending the TB epidemic by 2030 is among the health targets of the Sustainable Development Goals.[1] As per latest WHO definition, Pre-XDR-TB is described as MDR TB with resistance to any fluoroquinolone, whereas XDR-TB is Pre XDR TB plus resistance to at least one of the remaining Group A drugs i. e, bedaquiline or linezolid or both.[2] The estimated incidence of MDR/RR-TB (as per the Global TB Report 2022) in 2021 for India was 119,000 (93,000–145,000). The global coverage of testing for resistance to fluoroquinolones remains much lower, at just over 50% worldwide and below 25% in the South-east Asia in 2020.[3]

Fluoroquinolones are DNA gyrase inhibitors and resistance develop via mutations in bacterial genes encoding DNA gyrase or topoisomerase IV. Mutations in *gyrA* locus predicts clinically relevant levels of resistance to ciprofloxacin along with cross resistance to other fluoroquinolones such as ofloxacin.[4] The widespread use of these drugs has led to the marked emergence of fluoroquinolone resistant *Mycobacterium tuberculosis*.[5]

Rapid and accurate detection of MDR/XDR-TB is of importance as this allows for optimizing therapy and reducing transmission. This is best achieved using molecular tests such as CBNAAT and Line probe assey. Under NTEP, MTBDRsl Ver 2.0 assay is done for both first line and second-line drug resistance detection. Study done by Sethi S et al found that among FQ-resistant isolates, 18 different types of banding pattern corresponding to mutations were observed.[6] The commonest banding pattern appeared to be MUT3C followed by gyrA MUT1 and gyrA MUT3A respectively.[6] In this background, this study was done to find out the distribution and pattern of drug resistant causing mutations among FQ & second-line aminoglycosides (SLA) in *M. tuberculosis* isolates and to find out correlation, if any, between drug resistance conferring mutations and various patient related parameters.

2. Methods

This was a cross-sectional and observational descriptive study carried out at our centre under the National Tuberculosis Elimination Programme. Subjects with resistance to both first line and second line anti-tuberculosis drugs from 2020 to 2022 were included in the study. Patients who were resistant only to first line drugs and drug sensitive TB patients were excluded from the study. Patients identified as rifampicin resistant tuberculosis on CBNAAT (Gene Xpert, Cepheid) and/or first line LPA were further subjected to second-line LPA to identify additional resistance to Fluroquinolones (Levofloxacin & Moxifloxacin) and second line aminoglycosides (Amikacin, Ofloxacin and Kanamycin). Sputum samples were used for pulmonary TB and fine needle aspirate of lymph node or cold accesses were used for extrapulmonary TB. DNA extraction was done by denaturation by Sodium Hydroxide and centrifugation. DNA amplification was done by Taq DNA Polymerase using polymerase chain reaction followed by DNA hybridisation following the manufacturer's instructions. LPA which is a DNA strip-based test, helps to study drug resistance profile of MTBC strain by interpreting a pattern of bands that corelate with immobilized probes that are attached (or hybridized) to DNA amplification products. The clinical profile of each patient including demographic details, weight, height, past history of TB, HIV status, random blood sugar etc were collected from hospital medical records in MDR TB clinic and Nikhshay portal by Govt. of India. The data analysis was performed using Epi info version 7.2.1.0. Statistical significance was established at p-value of 0.05. The study was started after obtaining institutional ethical committee approval.

3. Results

The study had a target population of 250 rifampicin resistant isolates. After second line LPA, the final study sample included 123 fluoroquinolone resistant isolates including 14 isolates with additional second line aminoglycosides drug resistance. There were 78.0% (96/123) male patients and 21.9% (27/123) female patients. The mean age of distribution was 35.67 ± 4.6 years with mean age of male and female subjects of 37.2 ± 5.1 years and 20.6 ± 5.7 years respectively. 41.6% of male patients were in the age group 20–34 years where as among females, 37% patients were from the age group <19 years (Fig. 1). Out of total 123 subjects, 97 (78.8%) were from rural area whereas 26 (21.1%) were from urban area.75.6% (93/123) of the subjects were underweight with BMI< 18.5 and 21.9% (27/123) were in normal range and 2.4% (3/123) were overweight. Among 54.8% (51/93) of the underweight subjects (BMI< 16kg/m^2), 24% (22/93) were moderate underweight and 21.5% (20/123) were mildly underweight in this study.117 were sputum samples, four were lymph node needle aspirate (3.2%) and two were cold abscess (1.6%). So, most of the patients were pulmonary (95.1%) whereas only 4.8% were extra-pulmonary patients (Table 1).

Among 123 patients with fluoroquinolone resistance, 9 (7.3%) patients had no previous history of anti-tuberculosis therapy (ATT) and 114 (92.6%) had previous history of ATT. Among 127 FQ sensitive patients, 25 (19.6%) had no history of anti-tuberculosis therapy and 102 (80.3%) had previous history of ATT. This difference was statistically significant (p-0.004). Among 14 patients with second line aminoglycoside (SLA) resistance, 9 (64.2%) had history of anti-tuberculosis therapy and 5 (35.7%) had no history of ATT (Table 2). Liquid Culture DST to moxifloxacin was done in all patients with FQ resistance on SLLPA. 82 isolates (66.6%) were resistant to moxifloxacin on pDST. 35 FQ resistant isolates on SLLPA were sensitive on DST and 6 samples showed no growth on culture.

Table 3 shows different mutations and their frequency among FQ and SLA resistant isolates. The most prevalent mutation in FQ resistant isolates was in *Gyr A*gene (99.5%). The most common mutation detected was WT3 locus seen in 85 (69%) samples followed by MUT 3C locus seen in 50 (40%) samples. In Gyr B gene, the only mutations detected was present at WT1 locus seen in one patient. Hetero-resistance in Gyr A was observed in 14/123 isolates (11.3%). Most frequent pattern of hetero resistance was MUT 3B (D94N/Y) plus MUT

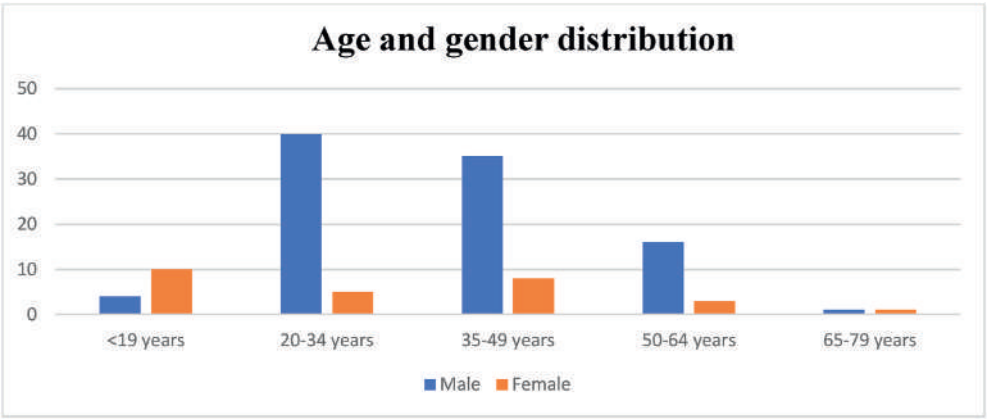


Fig. 1 – Figure showing distribution of study subjects according to age and sex.

3C (D94G) in 5 out of 123 (4%) patients. Triple mutations at MUT 3B, MUT3C and MUT3D were observed in 3 isolates (3/123, 2.4%) (Table 4).

The most common mutation among male patients was Gyr A WT3 (63/96,65.6%) followed by Gyr A MUT3C (37/96,38.5%) whereas the most common mutation among 27 female patients was Gyr A WT3 (22/27,81.4%) followed by Gyr A MUT3C (13/27,48.1%). WT 2 mutations was significantly observed among urban population (9/26, 34.6%) as compared to rural population (21/97, 22%, p-value <0.001). MUT3B (10/26, 38.4%) and MUT3C (11/26, 42.3%) mutation was also observed with significant difference among urban population as compared to rural population with p values of 0.001 and 0.014 respectively (Table 5).

The most common FQ resistance gene mutation among extra-pulmonary drug resistant tuberculosis isolates was MUT3C (3/6, 50%) followed by WT3 mutation (2/6, 33.3%). Furthermore, WT 3 mutation was significantly associated with extrapulmonary tuberculosis. (p-value-0.001). The only Gyr B mutation in the present study was detected in

Table 1 – Demographic profile and type of samples.

Parameter	No. of patients	Percentage
Male (n=96,78.0%)		
<19	04	4.1%
20–34	40	41.6%
35–49	35	36.4%
50–64	16	16.6%
>65	1	1.0%
Female (n=27,21.9%)		
<19	10	37.0%
20–34	5	18.5%
35–49	8	29.6%
50–64	3	11.1%
>65	1	3.7%
Rural	97	78.8%
Urban	26	21.1%
BMI		
<16	51	41.4%
16–16.9	22	17.8%
17–18.4	20	16.2%
18.5–24.9	27	21.9%
>25	3	2.4%
Sputum	117	95.1%
Lymph node aspirate	4	3.2%
Cold abscess	2	1.6%

Table 3 – Frequency of mutations among FQ and SLA resistant isolates (n = 123).

	Mutations	No. of patients	Percentage
FQ (n = 123)	Gyr AWT1	3	2.4
	Gyr AWT2	32	26.0
	Gyr AWT3	85	69.1
	Gyr AMUT1	26	21.1
	Gyr AMUT2	6	4.8
	Gyr AMUT3A	12	9.7
	Gyr AMUT3B	21	17.0
	Gyr AMUT3C	50	40.6
	Gyr A MUT3D	7	5.6
	GyrB WT1	1	0.8
	GyrB MUT1	0	0
	GyrB MUT2	0	0
SLA (n = 14)	Rrs WT1	1	7.1
	Rrs WT2	0	0
	Rrs MUT 1	1	7.1
	Rrs MUT 2	4	28.5
	Eis WT1	0	0
	Eis WT2	7	50.0
	Eis WT3	0	0
	Eis MUT 1	1	7.1

Table 2 – FQ and SLA resistance Drug resistance in relation to history of ATT.

	No h/o ATT	H/o ATT	p-value	Odd's ratio
FQ resistance (n = 123)	54 (43.9%)	69 (56.0%)	<0.001	2.41 (CI = 1.44–4.61)
FQ sensitive (n = 127)	83 (65.3%)	44 (34.6%)		
SLA resistant (n = 14)	5 (35.7%)	9 (64.2%)	0.14	2.28 (CI = 0.74–7.62)
SLA sensitive (n = 236)	132 (55.9%)	104 (44.0%)		

Table 4 – Frequency of hetero-resistance in GyrA locus (n = 14).

Mutation	No. of patients	Percentage
GyrAMUT3B + MUT3C	5	4.0
GyrAMUT 1 + MUT3A	3	2.4
GyrAMUT3B + MUT3D	2	1.6
GyrAMUT3A + MUT3C	1	0.8
Gyr A MUT3A + MUT3B + MUT3C	3	2.4
Total	14	11.3

pulmonary TB (sputum). No Second line aminoglycoside mutations were observed among 6 extra pulmonary drug resistant tuberculosis samples in the present study (Table 6).

Table 7 shows mutation profiles and their distribution according to body mass index. Gyr A MUT 3C was significantly associated with underweight patients as compared to patients having normal weight (46% vs 26%, p-value = 0.02). Gyr B, Rrs, Eis locus did not had enough sample size for statistical analysis.

Among 123 FQ and/or SLA resistant isolates, 70% (87/123) belong to age group of <40 years and 30% in the age group >40 years (36/123). WT3 mutation was observed to be significantly associated with the age group of <40 years (p value of 0.036) and MUT 3C was observed in significant number of patients with age group >40 years (52% vs 36%). All rrs and Eis gene mutation was seen in age group >40 years and none among those <40 years (Table 8).

4. Discussion

In this cross sectional, observational descriptive study, we analysed the resistance pattern and mutations associated with anti TB drugs (FQ and aminoglycosides) which are used in treatment of drug resistant tuberculosis. The highest burden was observed in men compared to women, who accounted for 74.7% of rifampicin resistant TB with additional FQ resistance which is higher than reported by Monir BB et al[7](27%) from Bangladesh. The present study shows a male-female ratio of 3.5:1 which is higher than reported by Shivekar et al[8] from JIPMER where the male-female ratio for incidence of tuberculosis was 1.9:1. MDR tuberculosis in the state of Rajasthan have a significant rural predominance. This was consistent with observation by Jangid et al[9] where 75% tuberculosis cases were from rural population of north west Rajasthan.

This study showed a significant high number of malnutrition among drug-resistant tuberculosis patients at our centre. In the present study, 75% had BMI<18.5 which was lower than

Table 6 – Mutation profile of pulmonary & extra-pulmonary patients.

Mutations	Pulmonary TB (n=117)	Extrapulmonary TB (n=6)	p- value
Gyr A WT 1	3 (2%)	0	0.337
Gyr A WT 2	30 (26%)	0	0.314
Gyr A WT 3	81 (69%)	2 (33.3%)	<0.001 (S)
Gyr A MUT 1	26 (22%)	0	0.431
Gyr A MUT 2	6 (5%)	0	0.687
Gyr A MUT 3A	12 (10%)	0	0.904
Gyr A MUT 3B	21 (18%)	0	0.560
Gyr A MUT 3C	44 (38%)	3 (50%)	0.959
Gyr A MUT 3D	5 (4%)	1 (16.6%)	0.775
Gyr B WT1	1 (0.8%)	0	–
Gyr B MUT1	0	0	–
Gyr B MUT 2	0	0	–
Rrs WT1	1 (0.8%)	0	–
Rrs WT2	0	0	–
Rrs MUT1	1 (0.8%)	0	–
Rrs MUT2	4 (3%)	0	–
Eis WT1	0	0	–
Eis WT2	7 (6%)	0	–
Eis WT3	0	0	–
Eis MUT1	1 (0.8%)	0	–

Table 5 – Comparison of Gender and residence of study subjects with mutation profile.

Mutations	Male (n=96)	Female (n=27)	Rural (n=97)	Urban (n=26)
Gyr A WT 1	2 (2%)	1 (3.7)	2 (2%)	1 (3.8%)
Gyr AWT 2	21 (21%)	9 (33)	21 (22%)	9 (34.6%)
Gyr A WT 3	63 (65%)	22 (81%)	63 (65%)	9 (34.6%)
Gyr A MUT1	19 (19%)	7 (25%)	22 (22%)	4 (15.3%)
Gyr A MUT2	4 (4%)	2 (7%)	2 (2%)	4 (15.3%)
Gyr A MUT3A	7 (7%)	5 (18%)	10 (10%)	2 (7.6%)
Gyr A MUT3B	14 (14%)	7 (26%)	11 (11%)	10 (38.4%)
Gyr A MUT3C	37 (38%)	13 (48%)	39 (40%)	11 (42.3%)
Gyr A MUT3D	4 (4%)	3 (11%)	4 (4%)	3 (11.5%)
Gyr B WT1	0	1 (3.7%)	1 (1%)	0
Gyr B MUT1	0	0	0	0
Gyr B MUT2	0	0	0	0
Rrs WT1	0	1 (3.7%)	1 (1%)	0
Rrs WT2	0	0	0	0
Rrs MUT1	0	1 (3.7%)	1 (1%)	0
Rrs MUT2	4 (4%)	0	4 (4%)	0
Eis WT 1	0	0	0	0
Eis WT2	3 (3%)	4 (14%)	7 (7%)	0
Eis WT3	0	0	0	0
Eis MUT1	0	1 (3.7%)	1 (1%)	0

Table 7 – Comparison of mutation profile and body mass index.

Mutations	Underweight (n=93, 75.6%) BMI:<18.5	Normal weight (n=27,22%) BMI:18.5–24.9	Obese (n=3, 2.4%) BMI>25	p-value
Gyr A WT 1	3 (3%)	0	0	0.609
Gyr A WT 2	20 (21%)	9 (33%)	1 (33%)	0.432
Gyr A WT 3	68 (73%)	15 (55%)	2 (66%)	0.220
Gyr A MUT 1	17 (18%)	9 (33%)	0	0.160
Gyr A MUT 2	5 (5%)	1 (3.7%)	0	0.868
Gyr A MUT 3A	12 (13%)	0	0	0.117
Gyr A MUT 3B	17 (18%)	4 (15%)	0	0.667
Gyr A MUT 3C	43 (46%)	7 (26%)	0	**0.026**
Gyr A MUT 3D	5 (5%)	2 (1.6%)	0	0.841
Gyr B WT1	1 (1%)	0	0	0.850
Gyr B MUT1	0	0	0	–
Gyr B MUT2	0	0	0	–
Rrs WT1	1 (1%)	0	3 (100%)	O.801
Rrs WT2	0	0	0	–
Rrs MUT1	1 (1%)	0	0	0.850
Rrs MUT2	4 (4%)	0	0	0.513
Eis WT 1	0	0	0	–
Eis WT2	7 (7.5%)	0	0	0.310
Eis WT3	0	0	0	–
Eis MUT 1	1 (1%)	0	0	0.850

Bold value signifies statistical significance.

data from the study by Singla et al[10] done in NIRTD, New Delhi in 2017 who found 93.9% patients having BMI in underweight category. Furthermore, 54% of total study subjects were severely underweight with BMI<16. Most of the MDR patients were pulmonary (95%) whereas only 5% were extra-pulmonary patients which was comparable to 2% extra pulmonary extensively drug resistant TB reported by Sharma et al[11] from North India in 2017.

The most frequent mutation found in Gyr A gene was Gyr A MUT 3C corresponding to substitution of Aspartic acid by Glycine at codon 94 (50/123,40.6%). This was comparable with the study by Sunil Sethi et al[6] in which the most frequent mutation found was also MUT 3C (Asp94Gly) in 42.9% (139/324) isolates which causes high level levofloxacin and moxifloxacin resistance. The second most common mutation was GyrA MUT1 corresponding to substitution of (Ala90Val) in 26 isolates (10%) which was also comparable with the previous study by Sunil Sethi et al[6] in which second commonest mutation in Gyr A locus was found in MUT1 (58/324, 17.9%). One isolate (1/123, 0.8%) had absence of GyrBWT1 showing a low prevalence of GyrB mutation in this region. In the study by same author, frequency of Gyr B mutation was 3.1% with most

Table 8 – Mutation profile of <40 years and >40 years.

	Mutations	<40 years(n=87)	>40 years(n=36)	p-value
FQ (n = 123)	Gyr AWT1	2 [2%]	1 [3%]	0.627
	Gyr AWT2	23 [26%]	7 [19%]	0.347
	Gyr AWT3	65 [74%]	20 [55%]	**0.036**
	Gyr AMUT1	17 [20%]	9 [25%]	0.666
	Gyr AMUT2	5 [5.7]	1 [3%]	0.814
	Gyr AMUT3A	10 [11%]	2 [5%]	0.499
	Gyr AMUT3B	15 [17%]	6 [16%]	0.852
	Gyr AMUT3C	31 [36%]	19 [52%]	0.119
	Gyr A MUT3D	4 [4.5%]	3 [8%]	0.700
	GyrB WT1	0	1 [3%]	0.647
	GyrB MUT1	0	0	–
	GyrB MUT2	0	0	–
SLA (n = 14)	Rrs WT1	0	1 [3%]	–
	Rrs WT2	0	0	–
	Rrs MUT 1	0	1 [3%]	–
	Rrs MUT 2	0	4 [11%]	-
	Eis WT1	0	0	–
	Eis WT2	0	7 [19%]	-
	Eis WT3	0	0	–
	Eis MUT 1	0	1 [3%]	–

Bold value signifies statistical significance.

frequent being absent WT1. The most frequent mutation for SLA resistance was rrsMUT2 (4/14, 28.5%) in our study. This was a different observation from the study by Sethi et al in which rrs MUT 1 was the most frequent mutation (43%).

Hetero-resistance was observed in 14/123 isolates (11.3%). Triple mutations at MUT 3B, MUT3C and MUT3D were observed in 3 isolates (3/123, 2.7%). Hetero-resistance was also observed in 27/863 (3.1%) isolates by Sunil Sethi et al,[6] but none having triple mutation. Among the fluroquinolones resistant isolates, 3% (7/123) isolates had mutation in WT2 band of eis locus followed by only one mutation in MUT 1 band corresponding to low level Kanamycin resistance. Co-existence of mutation was also reported by Kabir et al from Pakistan where 4% (4/102) of the isolates had co-existence of mutations.[12] A quarter of *M. tuberculosis* study isolates can be hetero-resistant to FQ. Thus, hetero-resistance can be an indication for future complete resistance.[13]

In a study by Ghosh et al[14] from Bose Institute, Kolkata, mutations at the MUT3C occurred more frequently in drug resistant MTB. This was similar to the finding in this study where most common mutation was Gyr A MUT3C. Farhat et al[15] reports that most common mutations associated with ofloxacin and moxifloxacin resistance are D94G (Gyr A MUT3C) and D94Y (Gyr A MUT3B) mutations and GyrB N538D (Gyr B MUT 1) mutations. The present study showed a higher frequency of mutation in MUT3C band in Gyr A gene locus (20%) compared to a study in Poland by Rosales KS et al.[16] in 2012, in which only 5.9% mutations were detected in MUT 3C band out of 117 MDR-TB isolates. Previously, another European study by Bakula Z et al[17] reported the resistance rate of FQ among MDR TB strains has been estimated to be 26.1% which was lower than the rate observed in the present study (76.8%) and those resistant to both FQ and Second line injectable (XDR-TB) was 6.5% which is comparable to the data detected from our study (8.75%). This difference suggests regional differences in drug resistance mutation profile.

The most common mutation in Gyr A in a study of 52 FQ resistant isolates by Gardee et al[18] in South Africa was Gyr A MUT3C (44.2%) which was higher than the data from our study (20%). In a study by David Patrick Kateete et al[19] from Uganda in 2016 Asp94Gly mutation (MUT 3C) was detected in 06 (33%) followed by Ala90Val (MUT 1) in 03 (16%) subjects as which was also higher compared to 20% and 10% respectively in the present study. The widely used target for the detection of FQ resistance is the QRDR (Quinolone resistance determining region) of the GyrA gene. A study by Tania Matsui et al[20] from Brazil showed 44% of FQ-resistant isolates harboured the Asp94Gly (MUT3C) mutation which was higher than the data from our study. These observations emphasize the effect of mutations in GyrA locus, on the development of fluroquinolones resistance, and provide estimate the percentage of MDR TB, that is pre-XDR TB. The high prevalence of FQ resistance is an area of concern for the national programs and also indicates imprudent use of FQ.

The most common gene mutation among extra pulmonary tuberculosis isolates were MUT3C (3/6, 50%) followed by WT3 mutation (2/6,33.3%). This finding was different from Sharma SK et al[11] who reports MUT 3B as most common mutation in EPTB (8.2%).

The most common mutation among 96 male patients was Gyr A WT3 (63/96,65%) followed by Gyr A MUT3C (37/96,38%) whereas the most common mutation among 27 female patients was Gyr A WT3 (22/27,81%) followed by Gyr A MUT3C (13/27,48%). Among 123 FQ and/or SLA resistant isolates, 78.8% [97/123] patients were from rural areas and 21.1% [26/123] were from urban areas. WT 2 mutations was significantly observed among urban population as compared to rural population (p-value <0.001). MUT3B and MUT3C mutation was also observed with significant difference among urban population as compared to rural population with p values of 0.001 and 0.014 respectively. Several studies have shown increasing rate of MDR tuberculosis in urban settings[21]; however, we could not find mutation profile analysis among urban versus rural settings.

75% of subjects with FQ and/or SLA mutations were underweighted, 27% were normal weight and 2% were overweight. Gyr A MUT 3C was significantly associated with underweight patients as compared to patients having normal weight (46% vs 26%, p-value = 0.02). This was higher as compared to a study by Kamara et al[22] who reported 32% underweight, 31% normal weight and 2% overweight among MDR TB patients. Among 123 FQ and/or SLA resistant isolates, 70% (87/123) were in the age group of 0–40 years and 30% were in the age group >40 years (36/123). WT3 mutation was observed to be significantly associated with the age group of 0–40 years (p value of 0.036) and MUT 3C was observed in significant number of patients with age group >40 years (52% vs 36%). Mahamood et al[23] reports 58.5% MDR patients in the age group <40 years in a study from Pakistan.

5. Strengths

The present study compares the FQ and SLA drug resistance mutation pattern among previously treated tuberculosis patients and patients without previous ATT history in Western India for the first time. Correlation of drug resistant tuberculosis with social, demographic, malnutrition and other parameters were also studied for the first time.

6. Limitations

The study is limited by the lack of sequencing data for validation of several drug-resistant associated mutations. When samples contain bacteria that are both drug-susceptible and drug-resistant, LPA is less effective than traditional culture-based DST at detecting resistance. Moreso, there were less extra-pulmonary samples for mutation profile analysis and hence effect of mutations in extra-pulmonary tuberculosis could not be studied in detail. Small sample size is a limitation of our study.

7. Conclusions

The present study shows that history of anti-tuberculosis therapy is a risk factor for FQ drug resistance mutations. The

present study also concludes that wild type 3 mutation as most common wild type mutation followed by MUT3C hybridization corresponding to single point mutation of Aspartic acid to Glycine at codon 94 as most common mutation in Gyr A gene locus in *M. tuberculosis* causing high level levofloxacin and moxifloxacin resistance from this vary part of country. Additional molecular epidemiology investigations are needed to determine the relationship between the fraction of MDR-TB cases with certain strains and continuous transmission to better understand the epidemiology and transmission dynamics of MDR-TB.

Conflicts of interest

The authors have none to declare.

Acknowledgments

The authors acknowledge the staff of the Intermediary Reference Laboratory (NTEP) and State TB Cell for their skillful technical assistance and financial support in this study.

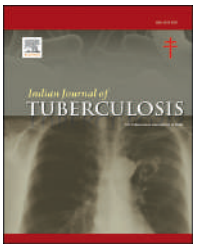
REFERENCES

1. World Health Organization. *Global Tuberculosis Report*; 2020. Geneva, [chapter 1] Available from: URL https://www.who.int/publications-detail-redirect/9789240013131.
2. *Guidelines for Programmatic Management of Drug Resistant TB in India*; 2021. Available from: URL https://ntep.in/node/3224/CP-pmdt-treatment-book.
3. World Health Organization. *Global Tuberculosis Report*; 2022 [Chapter 3]. Available from: URL https://www.who.int/tb-reports/global-tuberculosis-report-2022.
4. Abraham O, Naisuru A, Adeniyi KA. Mechanism of drug resistance in Mycobacterium tuberculosis. *Am J Biomed Sci*. 2020;7:5–10.
5. Pitaksajjakul P, Wongwit W, Punprasit W, et al. Mutations in the gyr A and gyr B genes of fluoroquinolone-resistant Mycobacterium tuberculosis from TB patients in Thailand. *Southeast Asian J Trop Med Publ Health*. 2005;36(4):228–237.
6. Sethi S, Agarwal P, Khaneja R, , et alYadav R. Second-line drug resistance characterization in *Mycobacterium tuberculosis* by genotype MTBDRsl assay. *J Epidemiol Glob Health*. 2020;10(1):42–45.
7. Monir BB, Sultana SS, Tarafder S, et al. 24 loci MIRU-VNTR analysis and pattern of drug resistance in pre-extensively drug resistant pulmonary tuberculosis in Bangladesh. *Infect Genet Evol*. 2022;102:105–120.
8. Shivekar S, Kaliaperumal V, Brahmacharry U, et al. Prevalence and factors associated with multidrug resistant tuberculosis in South India. *Nature*. 2020;10:175–179.
9. Jangid VK, Agrawal NK, Yadav GS, Pandey S, Mathur BB. Knowledge and awareness of the tuberculosis in tuberculosis patients at a tertiary care centre in north west Rajasthan, India. *Ntl J Community Med*. 2016;7(4):262–268.
10. Singla R, Raghu B, Gupta A, et al. Risk factors for early mortality in patients with pulmonary tuberculosis admitted to the emergency room. *Pulmonology*. 2021;27(1):35–42.
11. Sharma SK, Chaubey J, Singh BK, Sharma R, Mittal A, Sharma A. Drug resistance patterns among extrapulmonary tuberculosis cases in a tertiary care Centre in North India. *Int J TubercLungDis*. 2017;21(10):1112–1117.
12. Kabir S, Tahir Z, Mukhtar N, et al. Fluoroquinolone resistance and mutational profile of gyrA in pulmonary MDR tuberculosis patients. *BMC Pulm Med*. 2020;20:138–142.
13. Singh A, Zhao X, Drlica K. Fluoroquinolone heteroresistance, antimicrobial tolerance, and lethality enhancement. *Front Cell Infect Microbiol*. 2022;12, 938032.
14. Ghosh ANS, Saha S. Survey of drug resistance associated gene mutations in Mycobacterium tuberculosis, ESKAPE and other bacterial species. *Sci Rep*. 2020;10(1):895–897.
15. Farhat MR, Jacobson KR, Franke MF, et al. Gyrase mutations are associated with variable levels of fluoroquinolone resistance in *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2016;54:727–729.
16. Rosales-Klintz S, Jureen P, Zalutskayae A, et al. Drug resistance-related mutations in multidrug-resistant Mycobacterium tuberculosis isolates from diverse geographical regions. *Int J Mycobacteriol*. 2012;1(3):124–130.
17. Bakuła Z, Napiórkowska A, Zwolska Z, Bielecki J, Jagielski T. Second-line anti-tuberculosis drug resistance and its genetic determinants in multidrug-resistant Mycobacterium tuberculosis clinical isolates. *J Microbiol Immunol Infect*. 2016;49(3):439–444.
18. Gardee Y, Dreyer AW, Koornhof HJ, et al. Evaluation of the genotype MTBDRslversion 2.0 assay for second-line drug resistance detection of *Mycobacterium tuberculosis* isolates in South Africa. *J Clin Microbiol*. 2017;55:791–800.
19. David Patrick K, Kamugeya R. Frequency and patterns of second-line resistance conferring mutations among MDR-TB isolates resistant to a second-line drug from, Somalia and Uganda. *Pul Med*. 2019;19:124–126.
20. Matsui T, Pinhata JMW, Rabello M, et al. Frequency of first and second-line drug resistance-associated mutations among resistant *Mycobacterium tuberculosis* clinical isolates from SãoPaulo, Brazil. *Mem Inst Oswaldo Cruz*. 2020;115:200–205.
21. Almeida D, Rodrigues Camilla, Zarir F, et al. Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. *Clin Infect Dis*. 2003;36:152–154.
22. Kamara RF, Saunders MJ, Sahr F, et al. Social and health factors associated with adverse treatment outcomes among people with multidrug-resistant tuberculosis in Sierra Leone: a national, retrospective cohort study. *Lancet Global Health*. 2022;10(4):543–554.
23. Mahmood N, Abbas Shahzada N, Faraz N, et al. Mutational analysis of GyrB at amino acids: G481A & D505A in multidrug resistant (MDR) tuberculosis patients. *J Infect Public Health*. 2019;12(4):496–501.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Assessment of risk factors associated with drug-resistant tuberculosis in pulmonary tuberculosis patients

Chanda Vyawahare, Sahjid Mukhida, Sameena Khan, Nageswari R. Gandham, Sriram Kannuri, Shalini Bhaumik*

Department of Microbiology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra, 411018, India

ARTICLE INFO

Article history:
Received 4 January 2023
Received in revised form
10 May 2023
Accepted 24 July 2023
Available online 27 July 2023

Keywords:
Drug resistant tuberculosis
Drug sensitive tuberculosis
Risk factors
Nutritional level

ABSTRACT

Introduction: Tuberculosis remains a global health problem worldwide and the risk progression of Tuberculosis to Drug Resistant Tuberculosis is influenced by various factors. These include immunocompromised status, past history of tuberculosis, life style and nutritional level. Hence, identifying the population at risk of multidrug-resistant tuberculosis is essential and may help in developing appropriate case-finding strategies. Therefore, the present study was designed to study the contributing risk-factors associated with Drug resistant Tuberculosis.

Materials and methods: In this prospective observational study, we assessed 189 Pulmonary tuberculosis diagnosed patients during the period of 2 years at government recognized tertiary care centers. Data was collected from all these patients checked to investigate risk factors associated with Drug resistant tuberculosis development by multivariant analysis.

Results: Of the 189 participants, 36 were diagnosed with drug resistant tuberculosis and 153 with drug sensitive tuberculosis. Factors associated with drug resistant tuberculosis include low-weight (OR 8.50; $p = 0.0008430991$), low-BMI ($p = 0.0000527166$), lower economic status (OR-2.1351; $p = 0.048608696$) and tobacco (OR-4.5192; $p = 0.0023003189$) were found clinically and statistically significant in development of drug resistant tuberculosis. Binary logistic regression was performed to ascertain the effects of various statistically significant factors. Drug resistant tuberculosis patients were 7.77 times more likely to be tobacco users than drug sensitive tuberculosis.

Conclusions: Our study suggests that, there is a compelling and urgent need for increasing public awareness, initiating better nutrition and food programs, regular screening, and better management & control of MDR-TB.

* *Corresponding author*. Department of Microbiology, Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, Maharashtra, 411018, India. Tel.: +91 9974770469.
E-mail address: drssmukhida@rediffmail.com (S. Mukhida).

https://doi.org/10.1016/j.ijtb.2023.07.007

1. Introduction

Tuberculosis (TB), and in particular multidrug-resistant Tuberculosis (MDR-TB) caused by *Mycobacterium tuberculosis* (MTB) is a serious health concern that threatens the various precautionary efforts and curative initiatives designed to stop this deadly infection.[1] Drug-resistant tuberculosis (DR-TB) has been steadily on the rise since 1999, according to the world-wide surveillance project, which tracked occurrences of the new TB cases from 1999 to 2002. According to the recent report by World Health Organization (WHO), MDR-TB cases constitute about 18–21% of the cases in spite of being already treated by anti-TB drugs and 3–4% of freshly diagnosed untreated cases of TB.[2,3] Therefore, in countries that contribute to the majority of TB cases, limiting the transmission from infectious TB cases remains a key goal of tuberculosis control campaign, in addition to offering appropriate treatment and lowering mortality.[1,2,4]

The likelihood of developing an active disease after being exposed to tubercle bacilli is a combination of both extrinsic and intrinsic risk-factors. Transmission is higher in areas of social mixing and overcrowding. Similarly, the time of exposure to such an infective individual may also be extended if there is a delayed diagnosis.

While a number of host-related intrinsic factors contribute to the development of infection into a life-threatening illness, the WHO has identified numerous risk factors for the emergence of DR-TB.[5–7]

Numerous earlier studies found risk factors for MDR-TB, such as including prior TB therapy, non-compliance of treatment, cavitation in the lungs, exposure to an active TB Patient, Human Immunodeficiency Virus (HIV) infection, impaired glucose tolerance, alcoholism, malnutrition, cigarette-smoking, younger age-groups, gender, foreign-born people, working in health care.[8,9] In addition to the social, behavioral, and co-morbid factors, the prevalence of DR-TB is also influenced by aspects of sociodemographic parameters such regular earnings, domicile, and number of siblings in the family.

The spread of MDR-TB has been extensively hampering the TB control programs. This is due to the difficulty in treatment of such cases since typical short-course chemotherapy (DOTS) is somewhat effective in these patients and second-line medications are less effective and more harmful than first-line therapies.

As a result, determining the groups of individuals in danger for MDR-TB is vital and may aid in designing effective case-finding approaches. However, as most risk factors varied in their relations with MDR-TB across different geographical areas. There is number of research materials on MDR-TB, however there are very few studies that link risk factors. In light of this, the current study is intended to review the risk variables that contribute to DR-TB.

2. Materials and Methods

2.1. Type and duration of study

Cross-sectional study conducted for 2 Years (during November 2020–October 2022).

2.2. Place of study

National Tuberculosis Elimination Program (NTEP) Approved CBNAAT Centre of our tertiary care center.

2.3. Sample size

All Pulmonary Tuberculosis (PTB) detected patients (189) during the study period.

2.4. Inclusion-criteria

All PTB-diagnosed patients by a rapid molecular test including DS-TB and DR-TB.

2.5. Exclusion-criteria

1) All PTB not detected patients 2) Extra Pulmonary tuberculosis patient.

2.6. Process

Total 646 pulmonary specimens (Sputum and Broncho-Alveolar-Lavage) from presumptive PTB patients were processed by rapid molecular diagnosis test. Samples were processed as per the manufacturer's instructions and NTEP guidelines for testing.[10] Reporting was given in view of MTB detected/Not detected along with Rifampicin Resistance detected/not detected. All MTB-detected patients were referred to an in-house DOTS center for further treatment. All patients' data were collected about their co-morbidity, immunocompromised status, lifestyle, past correlation with TB, and nutritional level on below risk-factors, and respective data were collected. (Table 1).

2.7. Data collection

Standard Performa was prepared and patients' responses were notified in the respective form. Prepared the master sheet using Microsoft Excel for further analysis.

Table 1 – List of risk factors assessed during the study.

Co-morbidity/ Immunocompromised status	Life style	Past correlation with TB	Nutritional level
HIV	Tobacco addiction	Past history of TB	Weight
Diabetes	Alcohol addiction	Drug defaulter	Body Mass Index (BMI)
SARS Covid-19	Smoking	Family history	
	IV Drug Abuser	Contact with MDRTB	Economy status

2.8. Statistically analysis

Collected data analysed statistically by EpiInfo v7.2.5.0 (Dean AG, Arner TG, Sunki GG, Friedman R, Lantinga M, Sangam S, Zubieta JC, Sullivan KM, Brendel KA, Gao Z, Fontaine N, Shu M, Fuller G, Smith DC, Nitschke DA, and Fagan RF. EpiInfo™, a database and statistics program for public health professionals. CDC,Atlanta,GA,USA, 2011). Binary logistic regression was done using SPSS v23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)

2.9. Patient informed consent

Appropriate informed consent was taken from patients and/or their representatives before collecting their pulmonary specimens as well as their data collection.

2.10. Ethical approval

Ethical clearance is granted by the institutional ethical subcommittee for the study by the latter for I.E.S.C./270/2021 dated 23/12/2021.

3. Results

During the study period total 646 pulmonary specimens were processed for TB detection. Out of them, 189 (29.25%) patients were found positive for TB. Out of those 36 (19.35%), specimens were detected to be resistant to Rifampicin and/or Isoniazid by CBNAAT and/or Line Probe Assay. DR-TB patients group were include 23 MDR-TB patients, 8 Isoniazid mono resistant patients and 5 Rifampicin Resistant Patients.

All 189 patients' data were collected for risk-factor evaluation. Among them 55.02% (104) were male patients and 44.97% (85) were female patients. Similar number of male and female patients were found resistant to TB treatment (Table 2).

In the evaluation of co-morbidity and immunocompromised state, only 5 (2.65%) patients had HIV co-infection out of them 2 had been diagnosed with DRTB. We found 14 diabetic patients and 3 patients positive for Covid-19, but none of them were resistant to TB treatment. During the statistical analysis, none of them gave a significant correlation because their p-value is not <0.05 (Tables 3 and 4).

Lifestyle evaluation is specifically based on their addiction to Tobacco, Alcohol, smoking, and Intravenous (IV) drug abuse. A total of 22 (11.64%) patients had an addiction to tobacco and 10 (45.45%) out of them were found resistant to TB treatment. Alcohol addiction and smoking habits were present in 10 (5.29%) and 9 (4.76%) patients respectively and two among each were also found resistant. None of our patients had an IV drug-using history. As per the statistical analysis, Tobacco addiction had a significant correlation with DRTB due to their *p*-value is 0.0023 which is < 0.05.

In Past history or exposure to TB evaluation, our 10 (5.29%) patients were previously diagnosed with TB but only 1 (10%) of them had been diagnosed with DRTB. In previously diagnosed and treated patients only one patient was a drug defaulter who stopped the treatment after initiation but was not detected with DRTB. Our 6 (3.17%) patients had close contact with MDR-TB patients but only 1 (19.13%) was diagnosed with DRTB. Similarly, our 17 (8.99%) patients' family members were diagnosed with tuberculosis but only 3 (17.65%) patients with this family history were diagnosed with DRTB. During the statistical analysis, none of them gave a significant correlation because their *p*-value is not <0.05.

In nutritional level indicated risk-factor evaluation, low-weight (≤45 kg) was found in 136 (71.96%) patients, and out of them, 34 (25%) were detected with DRTB. When we calculated their BMI, 138 (73.02%) patients had low-BMI (<18 kg/sqm), and all 36 DRTB patients were coming to the low-BMI category. Our 98 (51.85%) patients belonged to the lower economic class of family and 24 (24.49%) patients among them were diagnosed with DRTB. During the statistical analysis, all three factors (low-weight, low-BMI, and low-economy) had a significant p-value of

Table 2 – Demographic detail wise risk factors characteristics in Drug-resistant tuberculosis (DRTB) and Drug sensitive tuberculosis (DSTB) patients.

Charateristics	Drug-resistant tuberculosis (36)	Drug-sensitive tuberculosis (153)	Total (189)	p-value
Sex				
Male	18	86	104	Chi-square=0.452
Female	18	67	85	df=1, p=0.5016
Age (year)				
Mean ± SD	32.58 ± 14.00	36.79 ± 16.99	35.99 ± 16.51	U=2381.50, Z=1.262, p=0.2071
Median	28.5	32	31	
Weight (Kg)				
Mean ± SD	40.33 ± 7.50	43.37 ± 9.36	42.79 ± 9.09	U=2088.50, Z=2.256, p=0.0241
Median	40.5	43	42	
Economy level				
Lower	24	74	98	
Middle	12	79	91	
BMI (Kg/Sqm)				
Mean ± SD	16.41 ± 1.09	18.27 ± 2.34	17.91 ± 2.27	U=1473.50, Z=4.337,
Median	16.6	17.5	17.	p<0.001

Table 3 – Risk factor evaluation in Drug-resistant tuberculosis (DRTB) and Drug sensitive tuberculosis (DSTB) patients.

Risk factor Comorbidity/ immunocompromised state		Drug-resistant tuberculosis (DR-TB)	Drug sensitive tuberculosis (DS-TB)	Total
HIV	Positive	2 (40.0%%)	3 (60.0%)	5 (2.65%)
	Negative	34 (18.48%)	150 (81.52%%)	184 (97.35%)
Diabetes	Yes	0	14 (100%)	14 (7.41%)
	No	36 (20.57%)	139 (79.43%)	175 (92.59%)
Covid-19	Positive	0	3 (100%%)	3 (1.59%)
	Negative	36 (19.35%)	150 (80.65%)	186 (98.41%)
Life Style				
Tobacco	Yes	10 (45.45%)	12 (54.55%)	22 (11.64%)
	No	26 (15.57%)	141 (84.43%)	173 (91.53%)
Alcohol	Yes	2 (20%)	8(80%)	10 (5.29%)
	No	34 (18.99%)	145 (81.01%)	179 (94.71%)
Smoking	Yes	2 (22.22%)	7 (77.77%)	9 (4.76%)
	No	34 (18.88%)	146 (81.11%)	180 (95.24%)
IV drug abuser	Yes	0	0	0
	No	36 (19.05%)	153 (80.95%)	189 (100%)
Past correlation with TB				
Past History of TB	Yes	1 (10.0%)	9 (90.0%)	10 (5.29%)
	No	35 (19.55%)	144(80.45%)	179 (94.70%)
Drug Defaulter	Yes	0	1 (0.52%)	1 (0.53%)
	No	36 (19.05%)	152 (80.42%)	188 (99.47%)
Contact with MDR TB patients	Yes	1 (19.13%)	5 (80.87%)	6 (3.17%)
	No	35 (16.67%)	148 (83.33%)	183 (96.83%)
Family History of Tuberculosis	Yes	3 (17.65%)	14 (82.35%)	17 (8.99%)
	No	33 (19.19%)	139 (80.81%)	172 (91.01%)
Nutritional level				
Low weight	< 45 Kgs	34 (25.00%)	102 (75.00%)	136 (71.96%)
	> 45 Kgs	2 (3.77%)	51 (96.23%)	58 (30.68%)
Economy Status	Lower	24 (24.49%)	74 (75.51%)	98 (51.85%)
	Middle or upper	12 (13.19%)	79 (86.81%)	91 (48.15%)
Body Mass Index (BMI)	< 18 (Low)	36 (26.08%)	102 (73.91%)	138 (73.02%)
	> 18	0	51 (100%)	51(26.98%)
Total		***36 (19.05%)***	***153 (80.95%)***	***189***

HIV: Human immunodeficiency virus, TB: Tuberculosis, IV: Intravenous, MDR: Multi Drug Resistant

0.0008430991, 0.0000527166, and 0.0486086496 respectively, and hence, found statistically significant. Binary logistic regression was performed to ascertain the effects of Diabetes, Tobacco, Low-Weight, Low-BMI, and Low-Economy on the likelihood that participants have DR-TB. DR-TB patients were 7.77 times more likely to be tobacco users than DS-TB (Table 5).

Table 4 – All risk factors combine statistical analysis results including Odds Ratio, Chi-square (wherever applicable), and *p*-value (Either by chi-square or fisher exact whichever is applicable).

Sr. No	Risk factors	Odds Ratio	95% Confidence Interval	Chi-Square	p-value
1	Co-infection with HIV	2.9412	0.4730–18.2901	–	0.2420279517
2	Comorbidity: Diabetes	–	–	–	***0.0757319847***
3	SARS CoViD-19 Pneumonia	0.8457	0.0957–7.4698	–	1.0000000000
4	Tobacco consumption	4.5192	1.7694–11.5426	–	***0.0023003189***
5	Alcohol addiction	1.0662	0.2166–5.2486	–	1.0000000000
6	Smoking addiction	1.2269	0.2440–6.1700	–	0.6815057461
7	Drug abuser	–	–	–	–
8	Previous Tuberculosis	0.4571	0.0560–3.7286	–	0.6903528087
9	TB treatment defaulter	–	–	–	1.000000000
10	Contact with DR TB patient	0.8457	0.0957-7.4698	–	1.000000000
11	Family history of Tuberculosis	0.9026	0.2451–3.3233	–	1.000000000
12	Low Weight (≤45kg)	8.5000	1.9638–36.7910	11.1439	***0.0008430991***
13	Low Body Mass Index	–	–	16.3478	***0.0000527166***
14	Lower Economy status	2.1351	0.9965–4.5749	3.8888	***0.0486086496***

*p-value <0.05 is significant for establishing the association between risk factors and DRTB

Table 5 – Multivariate logistic regression between statistical significant risk factors.

Variable	Group	DR-TB (%)	DS-TB (%)	Unadjusted OR (95 % CI)	Adjusted OR (95% CI)	P-Value
Diabetes	Yes	0	14 (100%)	–	–	0.998
	No	36 (20.57%)	139 (79.43%)			
Tobacco	Yes	**10 (45.45%)**	12 (54.55%)	4.5192 (1.7694–11.5426)	0.129 (0.036–0.459)	0.002
	No	26 (15.57%)	141 (84.43%)			
Low weight	< 45 Kgs	**34 (25.00%)**	102 (75.00%)	8.5000 (1.9638–36.7910	–	0.999
	> 45 Kgs	2 (3.77%)	51 (96.23%)			
Economy Status	Lower	**24 (24.49%)**	74 (75.51%)	2.1351 (0.9965–4.5749)	0.752 (0.311–1.821)	0.527
	Middle or upper	12 (13.19%)	79 (86.81%)			
Body Mass Index (BMI)	< 18 (Low)	**36 (26.08%)**	102 (73.91%)	–	–	0.999
	> 18	0	51 (100%)			

A logistic regression was performed to ascertain the effects of Diabetes, Tobacco, Low-Weight, Low-BMI, and Low Economy on the likelihood that participants have DR-TB. The logistic regression model was statistically significant, p<0.0001. the model explained 35.39% (Nagelkerke R2) of the variance in TB and Correctly classified 84.66% of cases. DR-TB patients were 7.77 (2.1799-27.7194) times more likely to be tobacco users than DS-TB.

* OR- Odds Ratio, CI- Confidence Interval.

4. Discussion

Our study provides information about significant risk factors associated with MDR-TB. Risk-factors, such as tobacco smoking, low-economic status, low-weight and lower BMI in relation with DR-TB in Western region of country were studied.

4.1. *Gender*

Gender correlation with DRTB has been studied by various researchers who have reported DRTB to be more prevalent in male patients as compared to females. Fox et al, Rifat et al, Elduma AH *et al*, Zhang C *et al*, and Prakash R *et al* reported 57%, 61%, 69.76%, 75%, and 76% of male DRTB patients respectively.[9,11–14] According to the study conducted by Caminero JA *et al*, DR-TB is more common in men than women because men are more likely to use drugs, smoke, and drink alcohol.[15] However, female predisposition for DRTB has been noted in few other studies.[9,16] In the present study, we found an equal number of male and female patients with DSTB and DRTB and no significant variation in genders which is similar to study done by Eldumaa AH *et al*.[11]

According to a European study, women are more compliant with treatment than men are, which lowers their chance of getting insufficient therapy and hence poor outcome.

4.2. *Age*

In our study, TB infection with development of DRTB was more in young children and geriatric patients. The mean age of DRTB was 32.58 years which is lower as compared to the mean age of DRTB patients (35.3 years) in the Sudan study and 38.3 years in an Indian study.[11,14] This shows that the age of DRTB development is decreasing and can even develop in young adult patients. Many studies have reported the age below 65 years to be associated with development of MDR-TB.[17,18] High activity levels and mobility in younger people increases their chances of coming in contact with DR-TB patients. Young people usually have odd working periods that often adds irregularity of treatment hence leading to poor treatment compliance.[8]

4.3. *Co-morbidity/immunocompromised status*

4.3.1. *HIV*

Immune response is altered by conditions like HIV that increase the danger of disease progression and the development of drug resistance. However, impact of this risk-factor could vary depending on the local prevalence of HIV in study area population. HIV co-infection makes severity of TB worse and vice a versa. Cell-mediated host defence against TB is weakened by HIV infection which leads to higher risk of TB relapse, dissemination and drug resistance.[19]

As reported by WHO, HIV infection has been found to be a leading risk-factor for DR-TB. A study by Mesfin YM *et al* reported 24% more risk in HIV patients to develop DR-TB whereas few other studies did not get any significant correlation between DR-TB and HIV like us.[20–24]

4.3.2. *Diabetes*

The role of Diabetes, as a risk-factor for the MDR-TB development still remains debateable. Several prior studies have reported co-morbid condition of DM with TB to be 2.1 to 8.8 times more at risk of developing DR-TB.[9,25] Studies conducted in Mexico, Georgia, and Israel have confirmed the higher risk of DR-TB in co-morbid patients.[26–28] Meta analysis done by Tegegne BS. *et al* which included 24 observational studies from 15 different countries, revealed DM to have a significant association with MDR-TB with statistical analysis value of Odds Ratio = 1.97 and *p*-value = 0.031.[29]

On the contrary, Bruker et al study and various other studies did not find any increased risk of DR-TB among TB cases with DM.[29–31] In current study, none of our DRTB patients had DM but *p*-value was found to 0.0757 which cannot be ignored as <0.1.

4.3.3. *Covid-19*

During the covid-19 pandemic, we tried to assess the patients for covid-19 and DRTB for any correlation but we did not find anything significant.

4.4. *Lifestyle*

DR-TB was 1.57 times more likely to advance in TB patients with a history of smoking exposure than in TB patients without a smoking history. Wang M *et al* did a systematic review for tobacco and DRTB correlation, and they established that tobacco smoking remains an individual risk-factor which has an increased risk of DRTB infection in a person.[2] In the current study 10/22(45.45%) tobacco user and 2/9 (22.22%), smokers had been diagnosed with DRTB. Statistical analysis shows ***p***-value of 0.002 which is significant to establish it as an important risk-factor. Similar findings were reported in Prakash R *et al*, Gomez–Gomez A *et al*, Fregona G *et al*, and Rajendran M *et al* studies.[14,27,32,33] Promoting smoking cessation programs and their regular monitoring may prove to be an active way to decrease development of DR-TB, TB relapse, and secondary TB transmission.

An Ethiopian study by Desissa F *et al* showed high association between alcohol consumption and MDR-TB occurrence.[22] WHO and other several reports, indicated that alcohol addiction is one of the risk-factor in developing MDR-TB due to poor treatment adherence and weakened immune responses. Nevertheless, various studies have found alcohol consumption as significant population-level risk-factor in development of MDR-TB we could not find any noteworthy association between them.[33–36] Similarly, none of our patients were found to be IV drug abuser.

4.5. *Past correlation with TB*

Past treatment emerges as the most prevalent and active factor for the development of DRTB.[2] This may be due to the suboptimal treatments or interruption in following the proper treatment protocols. As per global tuberculosis report published by WHO in 2021, only 3–4% of newer TB cases are MDR-TB while 18–21% of MDR-TB cases belong to previously treated TB patients.[3] This confirms the report by various studies which indicate that previously treated patients have around 8–10 times more chances of developing DR-TB as compared to others.[36–38]

Though we did not find any significant association between TB in family members, past TB history, drug defaulter, and contact with MDR-TB patients with DR-TB in our patients. In our study, only one patient out of 10 was having a previous history of being treated with TB drugs and detected DRTB. Also, 6 patients had a contact history with MDR-TB patients and only one of them was detected with DR-TB detected.

4.6. *Nutritional factors*

In the present study, more than 70% of patients had low-weight and Low-BMI while more than 50% found with low-economic status. All 36 DRTB patients were coming to the low-BMI category. On statistical analysis, all three factors had a significant *p*-value. This finding is well correlated with many other studies.[11,27,39–42] In a Yemen study by Jaber AAS *et al*, it was found that patients with weight <40 kg, were related with poor treatment results.[43]

Patients with low body-weight may be related to their weakened immune systems, which may cause TB to manifest more slowly. In these DRTB patients, programs for supply of nutritious food which may increase their weight along with immune status and results in successful treatment.

Among the above discussed various risk-factors, only few factors have statistical correlation, but this does not make rest of the factors less significant in importance for existence of DRTB. These results can differ and vary according to the epidemiology of the disease and the social, health, and economic status of the people.[44,45]

Further research and studies are required to include various other risk-factors such as marital status, unemployment, low education, residence in rural area, etc.

5. Conclusion

The findings of this study provide information regarding the risk factors for the development of DRTB in patients in the Western part of the country. Risk factors such as tobacco smoking, economic status, low weight, and low BMI show statistical association with rate of DRTB. Assessing the present situation, educating the public, diagnosing patients, and selecting a course of treatment for patients, enhancing drug along with initiating proper nutrition can help in the improved management and control of MDR-TB. Active tobacco/smoking cessation program for all PTB diagnosed patient (whoever is addicted) at the time enrolment in DOTS is initiated by NTEP and National Tobacco Control Program which help to decrease the DR TB development. To provide nutritious food to TB patient, direct benefit transfer under the Nikshya Poshan Yozana with help of Nikshya Mitra is implemented to get better treatment outcomes. We have to encourage the TB patient to get increased benefits from the government scheme to reduce the risk of MDR TB development. Doing so will undoubtedly aid the TB screening and management of MDRTB. These steps will serve as a turning point for controlling this fatal disease.

Author contribution

Dr. Chanda Vyawahare: Concept, Manuscript writing.

Dr. Sahjid Mukhida: Principle investigator, Data collection, Data analysis and follow up work, Manuscript writing.

Dr. Sameena Khan: Sample reporting, revision of content.

Dr. Nageswari R. Gandham: Drafting of article, revising it critically for important intellectual content.

Dr. Sriram Kannuri: Microbiological work done,

Dr. Shalini Bhaumik: Data collection.

All the authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Source(s) of support in the form of grants, equipment, drugs, or all of these

Financial support was received from the State TB Office, Pune, India (Jt. Director of Health Services (Leprosy & TB) Grant numbers: Jt. DHS/TB&L/Desk-NTEP/Thesis/14096-503).

Submission declaration

All authors are conforming that this study work is not published, accepted for publication or submitted in any journal for publication.

Conflicts of interest

The authors have none to declare.

Acknowledgements

We would like to extend our thanks to joint Director of Health Services (Leprosy and TB), Pune, Maharashtra India and Medical Officer Health, Corporation integrated health and family welfare society(TB), PCMC Pune, India for providing funds and support for study.

REFERENCES

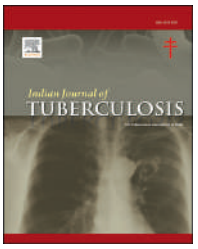
1. Liu Q, Li W, Xue M, et al. Diabetes mellitus and the risk of multidrug resistant tuberculosis: a meta-analysis. *Sci Rep*. 2017;7(1):1090.
2. Wang MG, Huang WW, Wang Y, et al. Association between tobacco smoking and drug-resistant tuberculosis. *Infect Drug Resist*. 2018;11:873–887.
3. *Global Tuberculosis Report 2021*. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
4. Kannuri S, Mirza S, Misra RN, et al. Role of cartridge-based nucleic acid amplification test in diagnosing extrapulmonary tuberculosis. *Med J DY Patil Vidyapeeth*. 2022;15(6):912–915.
5. Narasimhan P, Wood J, Macintyre CR, Mathai D. Risk factors for tuberculosis. *Pulm Med*. 2013;2013:828939. https://doi.org/10.1155/2013/828939. Epub 2013 Feb 12. PMID: 23476764; PMCID: PMC3583136.
6. World Health Organization. *Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*; 2014. Available from: http://apps.who.int/iris/bitstream/10665/75146/1/9789241548441_eng.pdf . Accessed October 12, 2022.
7. Mukhida S, Vyawhare C, Das P, Das NK. In need of new drugs: forgetting current use of the available drugs for tuberculosis. *Lung India*. 2023;40:289–290.
8. Sailo CV, Tonsing MR, Sanga Z, et al. Risk factors of tuberculosis in Mizoram: first report of the possible role of water source. *Indian J Tubercul*. 2022;69(4):675–681.
9. Rifat M, Milton AH, Hall J, et al. Development of multidrug resistant tuberculosis in Bangladesh: a case-control study on risk factors. *PLoS One*. 2014;9(8):e105214. https://doi.org/10.1371/journal.pone.0105214. PMID: 25136966; PMCID: PMC4138182.
10. Mukhida S, Vyawahare CR, Mirza SB, et al. Role of GeneXpert MTB/RIF assay for the diagnosis of cervical lymph node tuberculosis and rifampicin resistance. *Tzu Chi Med J*. 2022;34(4):418–422.
11. Elduma AH, Mansournia MA, Foroushani AR, et al. Assessment of the risk factors associated with multidrug-resistant tuberculosis in Sudan: a case-control study. *Epidemiol Health*. 2019;41:e2019014.
12. Fox L, Kramer MR, Haim I, Priess R, Metvachuk A, Shitrit D. Comparison of isoniazid monoresistant tuberculosis with drug-susceptible tuberculosis and multidrug-resistant tuberculosis. *Eur J Clin Microbiol Infect Dis*. 2011;30(7):863–867.
13. Zhang C, Wang Y, Shi G, et al. Determinants of multidrug-resistant tuberculosis in Henan province in China: a case control study. *BMC Publ Health*. 2016;16:42.
14. Prakash R, Kumar D, Gupta VK, et al. Status of multidrug-resistant tuberculosis (MDR-TB) among the Sahariya tribe of North Central India. *J Infect Public Health*. 2016;9(3):289–297.
15. Caminero JA. Multidrug-resistant tuberculosis: epidemiology, risk factors and case finding. *Int J Tubercul Lung Dis*. 2010;14(4):382–390. PMID: 20202293.
16. Lomtadze N, Aspindzelashvili R, Janjgava M, et al. Prevalence and risk factors for multidrug-resistant tuberculosis in the Republic of Georgia: a population-based study. *Int J Tubercul Lung Dis*. 2009;13:68–73.
17. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. *Thorax*. 2006;61:158–163.
18. Espinal MA, Laserson K, Camacho M, et al. Determinants of drug-resistant tuberculosis: analysis of 11 countries. *Int J Tubercul Lung Dis*. 2001 Oct;5(10):887–893. PMID: 11605880.
19. Sharma SK, Mohan A, Kadhiravan T. HIV-TB co-infection: epidemiology, diagnosis & management. *Indian J Med Res*. 2005;121(4):550–567. PMID: 15817963.
20. Mesfin YM, Hailemariam D, Biadglign S, Kibret KT. Association between HIV/AIDS and multi-drug resistance tuberculosis: a systematic review and meta-analysis. *PLoS One*. 2014;9(1):e82235.
21. Jitmuang A, Munjit P, Foongladda S. Prevalence and factors associated with multidrug-resistant tuberculosis at siriraj hospital, bangkok, Thailand. *Southeast Asian J Trop Med Publ Health*. 2015;46:697–706.
22. Desissa F, Workineh T, Beyene T. Risk factors for the occurrence of multidrug-resistant tuberculosis among patients undergoing multidrug-resistant tuberculosis treatment in East Shoa, Ethiopia. *BMC Publ Health*. 2018;18:422.
23. Singh A, Prasad R, Balasubramanian V, Gupta N. Drug-resistant tuberculosis and HIV infection: current perspectives. *HIV AIDS (Auckl)*. 2020;12:9–31. https://doi.org/10.2147/HIV.S193059. PMID: 32021483; PMCID: PMC6968813.
24. Saldanha N, Runwal K, Ghanekar C, Gaikwad S, Sane S, Pujari S. High prevalence of multi drug resistant tuberculosis in people living with HIV in Western India. *BMC Infect Dis*. 2019;19(1):391. https://doi.org/10.1186/s12879-019-4042-z. PMID: 31068153; PMCID: PMC6507020.
25. Pérez-Navarro LM, Fuentes-Domínguez FJ, Zenteno-Cuevas R. Type 2 diabetes mellitus and its influence in the development of multidrug resistance tuberculosis in patients from southeastern Mexico. *J Diabetes Complicat*. 2015;29:6.
26. Bendayan D, Hendler A, Polansky V, Weinberger M. Outcome of hospitalized MDR-TB patients: Israel 2000–2005. *Eur J Clin Microbiol Infect Dis*. 2011;30:5.
27. Gómez-Gómez A, Magaña-Aquino M, López-Meza S, et al. Diabetes and other risk factors for multi-drug resistant tuberculosis in a Mexican population with pulmonary tuberculosis: case control study. *Arch Med Res*. 2015;46:142–148. https://doi.org/10.1016/j.arcmed.2015.01.006.
28. Kikvidze M, Mikiashvili L. Impact of diabetes mellitus on drug-resistant tuberculosis treatment outcomes in Georgia-cohort study. *Eur Respir J*. 2013;42:P2826.
29. Tegegne BS, Habtewold TD, Mengesha MM, Burgerhof JG. Association between diabetes mellitus and multi-drug-resistant tuberculosis: a protocol for a systematic review and meta-analysis. *Syst Rev*. 2017;6(1):6.
30. Baker MA, Harries AD, Jeon CY, et al. The impact of diabetes on tuberculosis treatment outcomes: a systematic review. *BMC Med*. 2011;9:81.

31. Baghaei P, Tabarsi P, Abrishami Z, et al. Comparison of pulmonary TB patients with and without diabetes mellitus type II. *Tanaffos*. 2010;9:8.
32. Fregona G, Cosme LB, Moreira CMM, et al. Risk factors associated with multidrug-resistant tuberculosis in Espírito Santo, Brazil. *Rev Saude Publica*. 2017;51(0):41. https://doi.org/10.1590/S1518-8787.2017051006688. PMID: 28489185; PMCID: PMC5396494.
33. Rajendran M, Zaki RA, Aghamohammadi N. Contributing risk factors towards the prevalence of multidrug-resistant tuberculosis in Malaysia: a systematic review. *Tuberculosis*. 2020;122:101925.
34. Sinha P, Srivastava GN, Gupta A, Anupurba S. Association of risk factors and drug resistance pattern in tuberculosis patients in north India. *J Global Infect Dis*. 2017;9(4):139–145. https://doi.org/10.4103/jgid.jgid_167_16. PMID: 29302148; PMCID: PMC5750437.
35. Dutt R, Singh R, Majhi J, Basu G. Status of drug resistant tuberculosis among patients attending a tuberculosis unit of West Bengal: a record based cross-sectional study. *J Fam Med Prim Care*. 2022;11(1):84–89. https://doi.org/10.4103/jfmpc.jfmpc_576_21.
36. Mekonnen F, Tessema B, Moges F, Gelaw A, Eshetie S, Kumera G. Multidrug resistant tuberculosis: prevalence and risk factors in districts of metema and west armachiho, Northwest Ethiopia. *BMC Infect Dis*. 2015;15:461.
37. Pablos-Méndez A, Raviglione MC, Laszlo A, et al. Global surveillance for antituberculosis-drug resistance, 1994-1997. World health organization-international union against tuberculosis and lung disease working group on anti-tuberculosis drug resistance surveillance. *N Engl J Med*. 1998;338(23):1641–1649.
38. Mulu W, Mekonnen D, Yimer M, Admassu A, Abera B. Risk factors for multidrug resistant tuberculosis patients in Amhara National Regional State. *Afr Health Sci*. 2015;15(2):368–377.
39. Di Gennaro F, Pizzol D, Cebola B, et al. Social determinants of therapy failure and multi drug resistance among people with tuberculosis: a review. *Tuberculosis*. 2017;103:44–51.
40. Wang K, Chen S, Wang X, et al. Factors contributing to the high prevalence of multidrug-resistant tuberculosis among previously treated patients: a case-control study from China. *Microb Drug Resist*. 2014;20(4):294–300.
41. Padmapriyadarsini C, Shobana M, Lakshmi M, Beena T, Swaminathan S. Undernutrition & tuberculosis in India: situation analysis & the way forward. *Indian J Med Res*. 2016;144(1):11–20. https://doi.org/10.4103/0971-5916.193278. PMID: 27834321; PMCID: PMC5116882.
42. Sinha P, Ponnuraja C, Gupte N, et al. Impact of undernutrition on tuberculosis treatment outcomes in India: a multicenter, prospective, cohort analysis. *Clin Infect Dis*. 2023;76(8):1483–1491. https://doi.org/10.1093/cid/ciac915.
43. Jaber AAS, Ibrahim B. Health-related quality of life of patients with multidrug-resistant tuberculosis in Yemen: prospective study. *Health Qual Life Outcome*. 2019;17:142. https://doi.org/10.1186/s12955-019-1211-0.
44. Verma P, Jadhav SL, Dhone A, et al. Coverage, delays and implementation challenges of 'Direct Benefit Transfer' in the National Tuberculosis Elimination Programme – a mixed methods study. *Indian J Tubercul*; 27 May 2023. https://doi.org/10.1016/j.ijtb.2023.05.014.
45. Mukhida S, Das NK. When should we be able to divert the cost of tuberculosis to other diseases? *J Fam Med Prim Care*. 2023;12(5):1024–1025. https://doi.org/10.4103/jfmpc.jfmpc_214_23.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Factors associated with treatment adherence among pulmonary tuberculosis patients in New Delhi

Ilham Zaidi [a,*], *P.S. Sarma* [a], *Khalid Umer Khayyam* [b], *Quazi toufique Ahmad* [c], *V. Ramankutty* [d,e], *Gurpreet Singh* [a]

[a] *Achutha Menon Centre for Health Science Studies, SCTIMST, Trivandrum, Kerala, India*
[b] *Department of Epidemiology and Public Health. District TB Officer, National Institute of Tuberculosis and Respiratory Diseases, New Delhi, India*
[c] *World Health Organization, Country Office for India, New Delhi, India*
[d] *Amala Cancer Research Centre, Thrissur, India*
[e] *Health Action by People, India*

ARTICLE INFO

Article history:
Received 1 December 2022
Received in revised form
28 June 2023
Accepted 8 August 2023
Available online 11 August 2023

Keywords:
Pulmonary tuberculosis
Treatment adherence
Antitubercular drugs
DOTS
New Delhi

ABSTRACT

Background: TB is treated with a six-month course of four antimicrobial drugs, and nearly all cases of TB can be cured if the medications are given and taken correctly. Due to its prolong treatment plans, there can be reasons associated with non-adherence to treatment by TB patients. Hence, the present study aimed to explore the factors associated with medication adherence among TB patients.

Method: A cross-sectional descriptive survey was conducted among adult pulmonary tuberculosis patients enrolled under RNTCP (now NTEP) in New Delhi among 27 functional RNTCP districts. Around 200 TB patients who are enrolled in the Nikshay App and are also on treatment were considered. A structured questionnaire was prepared for the interview guide. Analysis was done using bivariate analysis, chi-square tests, and Fisher's exact tests.

Results: Among the total participants, 173 (86.5%) were adherent and the remaining 27 (13.5%) participants were non-adherent. The majority of the participants (91%) said they were able to follow the routine to the DOTS center, and 9% said they find it difficult to report to the DOTS center as per their schedule. Only 12.35% of non-adherent participants were seen among those who get regular reminders from their families to take medicines, as compared to 18.42% among those who did not get regular reminders from their families. More than one-fourth of the participants (25.9%) who report not getting necessary motivation from healthcare providers were non-adherent. Motivation by healthcare workers to follow drug schedules was found statistically significant to treatment compliance with a P-value of 0.0422.

Conclusion: TB is a curable disease; this belief has turned out to be a motivational factor for patients suffering from this disease. Studies have shown that faith in the efficacy of treatment helps adherence to TB treatment while other studies describe how patient

* *Corresponding author.*
E-mail address: ilhamasgher@gmail.com (I. Zaidi).
https://doi.org/10.1016/j.ijtb.2023.08.006

adherence was adversely affected by the belief that TB is incurable or the treatment is inefficient or that alternative treatment such as traditional medicine is better.

1. Introduction

India's significant endeavors to address TB through its public health services framework have prompted decreases in TB predominance and occurrence rates. All the more as of late, India's public medical services framework has likewise expanded its endeavors to address the development of Multidrug-resistant (MDR) TB.[1] TB is treated with a six-month course of four antimicrobial drugs, and nearly all cases of TB can be cured if the medications are given and taken correctly. However, adherence to the treatment is a major concern, and failure to do so contributes to MDR TB. Inappropriate medication usage, incorrect prescribing by healthcare providers, and low-quality medications can all lead to resistance to first-line drugs. These patients are then switched to second-line treatments, which are costlier, toxic, and require a longer treatment period of up to two years.[2]

Delhi is the Indian national capital regardless of having a more modest topographical region than some other States in India has the 10th highest notified TB patients at over 65,000 yearly TB incidences. The city also tops among all urban agglomerations in the country for having the highest migrant proportion and as per available literature, migrants fall in the high-risk category of developing active TB and also for non-adherence of treatment leading to drug-resistant TB.[3,4]

It had been established that one of the major reasons behind Tuberculosis being a public health emergency is that its long treatment action plan makes it difficult for patients to stay adherent.[5] Certain positive and negative reinforcement factors lead the patient to stay motivated, encouraged, and adherent to TB treatment, and some factors act as an obstacle for a patient to stay adherent to the treatment of TB. Hence, this study was proposed for identifying the factors associated with adherence to TB treatment among the population of New Delhi.

2. Methodology

A cross-sectional descriptive survey was conducted among adult pulmonary tuberculosis patients enrolled under RNTCP (now NTEP) in New Delhi. The study commenced in the month of March 2020 and ended in August 2020. It has been divided into 27 functional RNTCP districts.[4] TB patients from these districts who are enrolled in the Nikshay App and are also on treatment formed the sampling frame of the study. Exclusion criteria were patients less than 18 years of age, patients treated for extra-pulmonary TB, not responding despite three telecalls and those who are not consenting to the interview. As per RNTCP India annual report 2018, there were over 65 thousand incident cases from New Delhi, who were enrolled in the program.[4] To avoid the high risk of selection bias only the current beneficiaries enrolled in the program were kept as the target study population.

In a study, it was estimated that close to 50% of Indian TB patients are non-adherent to the treatment so frequency is kept at 50% (P = 50%), which would have been the same in case of no estimation of the prevalence at all. Therefore 50% frequency was kept optimal for the study.[6] A precision of 7.5% (d = 0.075) and a confidence interval of 95% were kept as per the suggestions by experts. Open Epi software was used for the calculation and the sample size obtained was 171. Another 10% non-respondent rate was added to 171, and it reached 190. It was further rounded to 200 and kept as the optimal sample size for the study.

Participants were contacted over the telephone to fix an interview appointment at the DOTS center. These participants were explained about the study using participant information sheets and their consent was taken on consent forms. Then they were interviewed by the principal investigator and their answers were marked on a hard copy of the interview schedule. These participants were handed over a copy of participant information sheet before the interview and a copy of answers to part IV of the interview schedule after the interviews. Only 20 interviews were possible before the COVID-19 Pandemic. As a precautionary measure, the Technical Advisory Committee and Institutional Ethics Committee of SCTIMST suggested taking all the remaining 180 interviews on telephone calls.

Confidentiality was best ensured during the interviews. A new sim card and mobile phone were issued for the purpose of calling the patients, the list of patient information along with the mobile phone and sim card were kept in the safe custody of DTO – NITRD Mehrauli, After the data collection was over the mobile phone was formatted in front of DTO- NITRD Mehrauli.

2.1. *Analysis*

Part 3 of the interview guide was analyzed based on its validated scale, and other components of the data were analyzed with their descriptive results. Outcome determining variables like Treatment compliance and convenience to treatment access among others. Five types of questions were asked to measure adherence.

i. Did you miss yesterday's medicine?
ii. Did you miss any medicine in the last one week?
iii. Did you miss any medicine in the last one month?
iv. Start you miss any medicine from the start of treatment?
v. Did you ever miss your medicine for 2 consecutive days?

Studies have defined non-adherents as a patient who misses more than or even equal to 10% of their medicines, and defaulters as those who discontinue their treatment for a longer duration [6]. The same definition for non-adherent is used in the study. Those who responded yes to the first and second questions were considered medically non-adherent since they fall under the category of missing 10% or more. People who responded yes to the fifth question were falling under the category of discontinuing for a long duration and therefore were also considered medically non-adherent. Those who responded yes to the third & fourth questions, but not to the first, second, & fifth questions were considered potentially non-adherent.

2.2. Statistical methods

The frequencies of dependent and independent variables were calculated to obtain descriptive results and a chi-square test was done to find the association of these variables with treatment adherence among pulmonary TB patients. Bivariate analysis using chi-square tests and Fisher's exact tests were done to study the relationship between the outcome and the predictor variables. Fisher's exact test was adopted when any of the cells in the table had a value less than 5. P values of less than 0.05 were considered for statistical significance. The statistical analysis was done using the software IBM SPSS Statistics Version 25 for Windows. Online platforms like socscistatistics.com were used when access to Institutional SPSS was not available.

3. Results

3.1. Patients characteristics

Out of 200 participants, 173 (86.5%) were adherent and the remaining 27 (13.5%) participants were non-adherent. Further, from 133 participants of the 18–30 age group, the majority of the participants (87.2%) were adherent and the remaining almost one-eighth of them (12.8%) were Non-adherent. And out of 67 participants from the age group above 30, the majority of the participants (85.1%) were adherent, and the remaining (14.9%) were non-adherent. More proportion of non-adherents was observed in the above 30 age group, as compared to the participants from the 18–30 age group. Only about one-tenth of the female participants (10.6%) were non-adherent and 16.03% of the males were non-adherent. The P value with gender factor by statistical analysis is 0.265. Adherence was observed higher in married people (96.23%) as compared to single/widowed participants who were adherent (75.53%). Association of marital status was found statistically significant to medication adherence with Fisher's Exact test statistic value (FET Value) of 0.0001 (Table 1).

As per the modified Kuppuswamy scale for 2019,[7] More than half of all the participants (52.5%) were from the lower class and more than one-fourth participants (27.5%) were from the lower middle class. Of the rest, 17.5% were from the upper middle class, and only 2.5% were from the upper class. In the broader category, the majority of the participants (80%) were from the lower class and lower-middle class, and only 20% were from the Upper & Upper Middle classes (Table 2).

Most of the interviewed participants (86%) were taking TB treatment for the first time. Out of the previously treated 28 participants, only one-fourth of them (7 participants) had completed the treatment and the remaining three-fourths of them (21 participants) had discontinued the treatment. Out of which 11 participants (52%) said they left the treatment because they felt cured and nobody suggested/motivated them to complete the treatment; the rest 48% of those who left the treatment was due to travel chaos, the unbearable expense of treatment, time constraints, and saturation from taking regular medications (Table 3).

More than half of the participants (61%) were called to the DOTS center once a week, and more than one-fourth of the participants (26.5%) were called to the DOTS center once a month. A small proportion of participants (11%) were called to the DOTS center on a daily basis, and very a handful of participants (1.5%) were called to the DOTS center less frequently than even once a month. The majority of the participants (91%) said they were able to follow the routine to the DOTS center, and 9% said they find it difficult to report to the DOTS center as per their schedule. Out of those, who find it difficult to follow up the routine at the DOTS center, almost one-fifth (22.2%) find it difficult to match the routine due because the location of the DOTS center is inconvenient for them; and two-fifths of the participants (38.9%) find the timings to DOTS center inconvenient, and another two-fifth of the participants (38.9%) find both the location as well as timings for the DOTS center inconvenient (Table 4).

3.2. Family's behavior toward TB patient

Non-adherence to TB treatment status in the patients who received positive care from the family was 12.5% and the patients who did not receive enough care from their family member was 15.6%. Around 159 participants got regular reminders from their families to visit DOTS centers for their timely treatment schedule. However, adherence to treatment percentage was 86.8% and among those 21 (13.2%) were not adhering to the treatment (Table 5).

Almost 47 participants did not get nutritious food from their families, among those 91.49% were adherent as compared to those who were getting nutritious food regularly only 84.97% of them were adhering to the treatment. Nevertheless, 15% of non-adherence to the treatment was seen among the participants who received nutritious food regularly (Table 5).

Among 85 participants who were no/not completely dependent on their families for financial support, 85.29% were adherent and 14.71% were non-adherent as compared to 85.22% adherent and 14.78% non-adherents among 115 participants who were completely dependent on their families (Table 5).

3.3. Healthcare providers

Only one participant claimed to be meeting the DOTS provider in their house and not in the DOTS center and was found to be adherent. In the remaining 199 participants who said to be

Table 1 – Adherence rate associated with patient's characteristics.

Age	Total	Adherent		Non adherent		P value
		Number	Percentage	Number	Percentage	
18 to 30	133	116	87.2%	17	12.8%	0.661
Above 30	67	57	85.1%	10	14.9%	
Gender and adherence						
Female	94	84	89.4%	10	10.6%	0.265
Male	106	89	83.96%	17	16.03%	
Association of Marital status with adherence						
Married	106	102	96.23%	4	3.77%	0.0001
Single/Widowed	94	71	75.53%	23	24.47%	

Table 2 – Socioeconomic status.

Variable	Category	Number of participants	Percentage
SES grade:	Upper class	5	2.5%
	Upper Middle class	35	17.5%
	Lower Middle Class	55	27.5%
	Upper lower class	101	50.5%
	Lower Class	4	2%
SES combined	Upper & Upper-Middle class	40	20%
	Lower-Middle, and lower class	160	80%

Table 3 – History and present status of TB treatment.

Variable	Category	Number of participants	Percentage
First time TB treatment	No	28	14%
	Yes	172	86%
If no, completed treatment previously? (N = 28)	No	21	75%
	Yes	7	25%
Reason for discontinuation: (N = 21)	Could not afford travel cost	2	9.6%
	Could not afford travel cost and medicines	1	4.8%
	Could not afford travel cost, felt cured and regular treatment was not suggested	2	9.6%
	Could not afford travel cost and no time from school	2	9.6%
	Depression and anxiety	1	4.8%
	Felt cured	1	4.8%
	Felt cured and regular treatment was not suggested	11	52%
	Regular treatment was not suggested	1	4.8%

meeting the DOTS provider at the DOTS center, 13.57% and 86.43% were found to be non-adherent and adherent, respectively. Fisher's exact test was performed to find the association, and the value came out as 1, which is not statistically significant (Table 6).

Less than one-eighth of the participants (11.02%) were non-adherent from those who were always asked about their well-being by the healthcare provider, which was a much lower proportion as compared to 17.8% of those who were not always asked by the healthcare worker about their health status (Table 6).

Of all the participants who said that the healthcare provider always motivates them to follow the drug schedule properly, 88.44% were found to be adherent, and the remaining (11.56%) were non-adherent, as compared to 74.1% adherent and 25.9% non-adherent among those who did not receive constant motivation from the healthcare provider to follow the drug routine. The association was found statistically significant with p value < 0.05 (Table 6).

4. Discussion

According to a previous study and the WHO Global TB Survey (2018), 10.0 million people were infected with tuberculosis in the year 2017. MDR-TB was found in 3.5% of new cases and 18% of previously treated cases around the world. For India, the figures are 2.8% and 12%, respectively. TB, even following quite a while of accessibility of treatment, is a challenge for public health personnel around the world. Constant exploration and evidence-based decision-making can be fundamental for tackling the issue of ensuring compliance with TB

Table 4 – Visits to DOTS centre and adherence.

Variable	Category	Number of participants	Percentage
Frequency of being called by the DOTS centre:	Daily	22	11%
	Monthly	53	26.5%
	More than a month	3	1.5%
	Weekly	122	61%
Able to follow the DOTS schedule:	No	18	9%
	Yes	182	91%
If no, reasons for missing out on schedule(N = 18):	Inconvenient location	4	22.2%
	Inconvenient timings	7	38.9%
	Both	7	38.9%
Missing out on medicine adherence:	No	138	69%
	Yes	62	31%
Non-adherence:	Adherent	138	69%
	Medically Non-Adherent	27	13.5%
	Potential Non-Adherent	35	17.5%

Table 5 – Role of family members in adherence to TB medication.

Care from Family	Total	Adherent		Non adherent		P value
		Number	Percentage	Number	Percentage	
Not enough care	64	54	84.38%	10	15.62%	0.546
Positive change	136	119	87.5%	17	12.5%	
Informed by family to visit DOTS centre						
No/Not always	41	35	85.37%	6	14.63%	0.812
Yes	159	138	86.8%	21	13.2%	
Nutritious food offered by the family						
No/Not always	47	43	91.49%	4	8.51%	0.333
Yes	153	130	84.97%	23	15.03%	
Financial dependence on family						
No/Not Completely	85	75	85.29%	10	14.71%	0.537
Yes	115	98	85.22%	17	14.78%	

Table 6 – Role of healthcare provider.

Meeting DOTS Provider	Total	Adherent		Non adherent		P value
		Number	Percentage	Number	Percentage	
DOTS centre	199	172	86.43%	27	13.57%	1
At home	1	1	100%	0	0%	
Healthcare provider ask about well being						
Always	127	113	88.98%	14	11.02%	0.176
Not always	73	60	82.2%	13	17.8%	
Motivation by Healthcare provider to follow the drug schedule						
Always	173	153	88.44%	20	11.56%	0.042
Not always	27	20	74.1%	7	25.9%	

treatment.[8] Therefore, the present study was done to aid in the identification of the factors determining the adherence or non-adherence to the treatment of TB patient effective management.

4.1. *Age and gender associations to TB patient's adherence to treatment*

In the present study, there are a higher proportion of male patients. It was observed that 12.8% of non-adherence was seen among the younger age group (18–30 years old) whereas almost 15% of non-adherence was found in the older age group i.e. 30 years and above age. In previous studies by Bagchi et al. and Furlan et al. showed similar results.[9,10] In a study done by Trivedi et al. in the year 2018, it was found that out of 133 patients, 84 (63.15%) were male and 49 (36.84%) were female. Among both genders, the most common age group was 21–30 years with 41 patients (30.82%) and the least common was pediatric TB (in age group <10 years) with 10 patients (7.51%).[12] This association of age and gender has to be explored further to find out such a biased distribution of the disease.

4.2. *Facilitator's role in adherence to TB treatment in patients*

There have been several studies done worldwide on factors leading to adherence and non-adherence to TB treatment, but

there are limited numbers of research from the region, and therefore there is a dearth of data. It has been found that several factors are responsible that act as positive and negative reinforcements for the patient to stay compliant with the treatment.[11]

4.3. Role of family care in adherence to TB treatment

Many studies have tried to measure and analyze the factors and there is a rich literature on the topic. Some commonly accepted factors which help people to stay adherent are the constant support and encouragement of the spouse, children, and other family members. Society and peer status also contribute to staying adherent to the treatment and social status is identified as an important factor for staying compliant with the treatment of TB.[2,6] This study also gave similar results, most of the participants were able to adhere to the treatment because of the timely reminder by their family members to visit the DOTS center. More than 85% of the participants claimed that they received care from their families and were provided good nutritious food to recover from TB. This encouraged them to not skip any of the appointments in the DOTS center. Now, nutritious food for TB patients in our study implies nutritional care and support provided for patients with tuberculosis all over India as per the guidelines that are issued by the Central TB Division, Directorate General of Health Services, Ministry of Health & Family Welfare Government of India.[14] The marital status of an individual positively impacts the person to keep him constant in taking the treatment as proved in this study. Similar results were obtained in a study done by Weigou et al., 2009 bivariate analysis showed that illiterate/uneducated, divorced/widowed, lacked health insurance, and migrants, were more likely to be non-adherent and similar results were seen in the present study.[5] However, 47% of the participants who were single (inclusive of widowed, divorced, and unmarried individuals) were found to have missed their DOTS appointments.

4.4. Barriers in adherence to TB treatment by patients

It had been established that one of the major reasons behind Tuberculosis being a public health emergency is that its prolonged treatment, is difficult for patients to stay adherent to. WHO defines adherence to TB treatment as staying compliant with the prescribed drug schedule.[2] Adherence has been measured in many studies across the globe by loyalty to the TB treatment and prescribed dose schedule.[2]

4.5. Accessibility to DOTS center

Distance from DOTS center, facilities to commute to the center, timings of center, and behavior attitude of healthcare providers have also been important factors to stay adherent to their treatment. Another major point discussed in studies is the saturation from staying adherent to the very long TB treatment, the chaotic procedure of taking so many medicines, and the adverse effects of anti-tubercular drugs which lead patients to miss or discontinue their treatment.[2,9]

Although the majority of the participants (91%) said they were able to follow the routine at the DOTS center, the problems of the other participants cannot be ignored. The study reveals that around 9% said they find it difficult to report to the DOTS center as per their schedule. Out of those, some find it difficult to follow up the routine at the DOTS center, almost one-fifth find it difficult to match the routine because the location of the DOTS center is inconvenient for them, almost two-fifths of the participants (38.9%) find the timings to DOTS center inconvenient, and another two-fifth of the participants (38.9%) find both the location as well as timings for the DOTS center inconvenient.

4.6. Role of healthcare providers in adherence to TB treatment

It was also revealed by the responses received by the participants that the DOTS providers do not give home visits. Now, this can be an alarming issue for discontinuation of treatment by the patient. It was thereby evaluated that non-adherence was more in the patients who did not receive attention from the healthcare provider. Either lack of counseling or motivation from the healthcare provider also contributed to the non-adherence to the treatment. This similar association was also proved in the study conducted in China. It stated good doctor-patient trust and communication are essential; to make the patient adhere to the treatment throughout the regime.[13]

Face-to-face interviews with the participants would have been preferable for the study, which is one of the limitations. Also, since the study was done during the lockdown, DOTS was not directly observed; medicines were distributed to patients even for months altogether. There is a chance of missing out on defaulters who discontinued the treatment and were not reached.

Under the Revised National Tuberculosis Control Program (RNTCP), DOTS has proved to be an effective strategy. The decline in mortality and increased adherence to therapy in India can be attributed to the implication of DOTS majorly. Based on the present study findings, a multi-sectoral involvement needs to be encouraged for capacity building of the healthcare workers ad providers which will indirectly ensure compliance with the treatment. Sensitivity among the public and educational campaigns on prevention/treatment should be strengthened to bring about TB elimination in India.

The government of India is putting forward numerous attempts to cut down the issues related to TB through updated plans and their execution in the nation over. Despite this, there is far to go to accomplish a huge decrease in the incidence rate and pervasiveness of TB in India. Factors like the absence of awareness and limited assets, the upsurge of MDR TB, untreated, unreported, and by and large carelessness towards treated adherence are significant difficulties. Infectious illnesses like TB can deceive anybody. Indeed, even inoculations do essentially nothing to diminish its effect. Bridging the gaps between availability, and accessibility along with constant support and involvement of family members and healthcare providers, we can expect a TB-free country.

The ongoing COVID pandemic in 2020 has likewise offered us a brilliant chance to make mindfulness about TB locally at different levels. The Global Tuberculosis Report 2022 was published by WHO based on statistics submitted by 202 nations and territories, covering more than 99% of the world's

population and TB cases. The COVID-19 pandemic, has interrupted or reversed the progress gained in the battle against TB up through 2019. It continues to have a detrimental impact on the diagnosis and treatment of tuberculosis, as well as the burden of the illness.[15] Essential TB services were not provided to many patients during the COVID-19 epidemic. As a result, the identification of newly confirmed cases of TB reduced in 2020 (5.8 million compared to 7.1 million in 2019).[15] This decline compared to before the COVID-19 pandemic points to an increase in undiagnosed and untreated TB cases, which will cause the community spread of infection and TB death.

5. Conclusion

TB is a curable disease; this belief has turned out to be a motivational factor for patients suffering from this disease. The study reveals that faith in the efficacy of treatment helps adherence to TB treatment. The findings also suggest that the provision of food and minimal financial support might facilitate absolute adherence to the treatment. Nevertheless, counseling might also facilitate adherence in the early phases of TB treatment along with consecutive follow-ups until treatment completes. During counseling side effects and pill burden should be addressed properly. Patients should be well informed about co-infection and concomitant treatment. Patients should be aware of the side effects, pill burden, and duration of treatment. Patients should be informed about the importance of social support along with the financial support provided by the Government of India through Nikshay Poshan Yojna (NPY) during the course of treatment.

For TB treatment adherence, age, sex, socioeconomic status, types of accommodations, malnutrition, and personal hygiene should all be considered. In the care of tuberculosis patients, knowledge and awareness about TB and its treatment, family support, DOTS services, and health workers play a significant role. According to the country's National Strategic Plan, the Indian government has set a goal of eliminating tuberculosis by 2025. To achieve this aim, improving patients' compliance with TB care and treatment adherence is the most crucial challenge for the country. As per the study done, The RNTCP has sustained the objectives (NSP case detection rates of over 70% and treatment success rates of over 85% nationally) since 2007, in line with global targets for TB control. To achieve the ultimate goal of TB control in India, the program will have to be sustained for many years to come. Continued decentralization of program management and implementation, ensuring financial support for the RNTCP, and mobilization of community participation in TB control efforts would facilitate the process.

Funding

None.

Conflicts of interest

All the authors declare there are no conflicts of interest.

Acknowledgment

The authors are grateful for the assistance and cooperation of the tuberculosis officers of Mehrauli, New Delhi, and all the medical and paramedical staff including treatment observers who participated in this work. We thank the patients who cooperated with the interview.

REFERENCES

1. Verma R, Khanna P, Mehta B. Revised national tuberculosis control program in India: the need to strengthen. *Int J Prev Med*. 2013 Jan;4(1):1.
2. World Health Organization. *Adherence to Long-Term Therapies: Evidence for Action*. World Health Organization; 2003 [cited 20 February 2020] https://apps.who.int/iris/handle/10665/42682.
3. *Census of India*; 2011. Drop-in-Article-No. 8: Migration. [cited 20 February 2020] https://censusindia.gov.in/nada/index.php/catalog/40447/download/44081/DROP_IN_ARTICLE-08.pdf.
4. India TB Report. *Revised National TB Control Programme Annual Status Report, Central TB Division, Director General of Health Services*. New Delhi: Ministry of Health and Family Welfare; 2018, 2018.
5. Xu W, Lu W, Zhou Y, Zhu L, Shen H, Wang J. Adherence to anti-tuberculosis treatment among pulmonary tuberculosis patients: a qualitative and quantitative study. *BMC Health Serv Res*. 2009 Dec;9(1):1–8.
6. Mekonnen HS, Azagew AW. Non-adherence to anti-tuberculosis treatment, reasons and associated factors among TB patients attending at Gondar town health centers, Northwest Ethiopia. *BMC Res Notes*. 2018 Dec;11(1):1–8.
7. Saleem SM, Jan SS. Modified Kuppuswamy socioeconomic scale updated for the year 2019. *Indian J Forensic Community Med*. 2019 Jan;6(1):1–3.
8. Giri OP, Giri VP, Nikhil N. Socio-demographic profile of MDR-TB and XDR-TB patients admitted in DR-TB centre, north India. *J Assoc Phys India*. 2019 Oct 1;67:61.
9. Bagchi S, Ambe G, Sathiakumar N. Determinants of poor adherence to anti-tuberculosis treatment in Mumbai, India. *Int J Prev Med*. 2010;1(4):223.
10. Furlan MC, Oliveira SP, Marcon SS. Factors associated with nonadherence of tuberculosis treatment in the state of Paraná. *Acta Paul Enferm*. 2012;25:108–114.
11. Gebremariam MK, Bjune GA, Frich JC. Barriers and facilitators of adherence to TB treatment in patients on concomitant TB and HIV treatment: a qualitative study. *BMC Publ Health*. 2010 Dec;10:1–9.
12. Rohit RT, Niranjan A, Pawan PA. Socio-demographic profile and outcome of TB patients registered at DTC Rewa of Central India. *Indian J Tubercul*. 2018 Apr 1;65(2):140–144.
13. Du L, Wu R, Chen X, Xu J, Ji H, Zhou L. Role of treatment adherence, doctor–patient trust, and communication in predicting treatment effects among tuberculosis patients: difference between urban and rural areas. *Patient Prefer Adherence*. 2020;14:2327.
14. Central TB Division. Directorate general of health services. In: *Ministry of Health and Family Welfare. Guidance Document: Nutritional Care and Support for Patients with Tuberculosis in India*New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare; 2017. https://tbcindia.gov.in.
15. Bagcchi S. WHO's global tuberculosis report 2022. *The Lancet Microbe*. 2023 Jan 1;4(1):e20.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Original article

Detection of drug-resistant *Mycobacterium tuberculosis* in pericardial fluid culture and its correlation with cartridge based nucleic acid amplification test and adenosine deaminase activity☆

Jyoti Bajpai [a], *Shubhajeet Roy* [b], *Suruchi Shukla* [c], *Akshyaya Pradhan* [d,*], *Surya Kant* [a], *Shobhit Shah* [d]

[a] *Department of Respiratory Medicine, Kasturba Chest Hospital, King George's Medical University, Lucknow, India*
[b] *Faculty of Medical Sciences, King George's Medical University, Lucknow, India*
[c] *Department of Microbiology, King George's Medical University, Lucknow, India*
[d] *Department of Cardiology, Lari Cardiology Centre, King George's Medical University, Lucknow, India*

ARTICLE INFO

Article history:
Received 29 August 2023
Accepted 22 September 2023
Available online 29 September 2023

Keywords:
Tuberculosis
Pericardial effusion
Culture
CBNAAT
Adenosine deaminase

ABSTRACT

Background: Pericardial effusion is the accumulation of fluid in the pericardial cavity. In nations with high tuberculosis (TB) load, TB is the most common cause of pericardial effusion. 1–2% of patients with pulmonary TB develop Pericardial TB worldwide. Multi-drug-resistant (MDR) TB, including extrapulmonary TB (EPTB) cases, are rising in number. Adenosine Deaminase (ADA) is an enzyme in lymphocytes and myeloid cells, which has certain immune functions in the body. ADA levels are increased in inflammatory conditions, like pleural, pericardial, or joint effusions, of bacterial etiology, granulomatous conditions, neoplasms, and autoimmune pathologies. TB is the only lymphocytosis involving disease with increased ADA levels. MDR EPTB is rare, but cases are on the rise, and tuberculous pericardial effusion is one such example. Hence, it is important to know the percentage of cases detected by a culture that can be identified by cartridge-based nucleic acid amplification test (CBNAAT), their resistance patterns, and to identify potential markers like ADA, which can help in early identification of cases. The objectives of this study were to identify the *Mycobacterium tuberculosis* (MTB) bacilli in culture, and correlate them with cartridge-based nucleic acid amplification test (CBNAAT) results and their drug-resistance, in the Pericardial tubercular effusion, and to find if Adenosine Deaminase (ADA) levels can be used as a predictor of the presence of MTB in pericardial fluid.

Methodology: We enrolled 52 patients with moderate to large tuberculous pericardial effusion, based on pericardial fluid analysis, CBNAAT, and culture methods, between January 2021 and December 2021.

☆ The institution at which the work was performed: King George's Medical University, Lucknow– 226,003, Uttar Pradesh, India.

* *Corresponding author*. Department of Cardiology, Lari Cardiology Centre, King George's Medical University, Shah Mina Shah Road, Chowk, Lucknow, 226003, India
E-mail address: akshyaya33@gmail.com (A. Pradhan).

https://doi.org/10.1016/j.ijtb.2023.09.014

Results: The mean age of the patients was 41.85 + 17.88 years, with a median of 38 years. Males made up 57.7% of the total patients. MTB was detected in 16 (30.8%) patients in the CBNAAT evaluations. 14 (87.5%) of the CBNAAT-positive TB patients were sensitive to Rifampicin, whereas the remaining 2 (12.5%) were resistant to Rifampicin on CBNAAT. MTB was found to be growing in 8 (15.38%) drug sensitivity test cultures. Out of these 8, 6 were sensitive to first-line drugs, whereas 2 were resistant to both Isoniazid and Rifampicin. The presence of cough was found to have a significant difference between CBNAAT-detected MTB positive and negative patients ($p = 0.020$), whereas an insignificant difference was found for the presence of hypertension, diabetes mellitus, obesity, dyspnea, or fever. There was also an insignificant difference between the number of patients positive for the Tuberculin skin test, between the two groups. ADA was significantly higher in the MTB-detected CBNAAT group (85.91 + 37.60U/L vs 39.78 + 24.31U/L, $p = 0.005$), whereas the total leukocyte count, lymphocytes, neutrophils, random blood sugar levels, and serum protein levels had no significant difference. The area under the Receiver Operator Curve (CBNAAT positive: dependent variable; ADA: test result variable) was 0.854 (null hypothesis rejected), with a standard error of 0.078.

Conclusions: Culture is the gold standard method to diagnose tuberculosis. Detection of MTB on pericardial fluid culture is very uncommon, though in our study, culture came out positive in 16% of patients, and 4% were resistant to rifampicin and isoniazid. Higher ADA levels in pericardial fluid are an indicator of tuberculous pericardial effusion.

1. Introduction

Pericardial effusion is defined as the collection of excess fluid in the pericardial sac which covers the heart, and is bounded by the two layers of the pericardium— the visceral and the parietal. Normally, the pericardial fluid is serous, and its volume ranges from 15 to 50 mL. However, due to various pathologies, the fluid may become transudative, exudative, or sanguineous (hemorrhagic) and might also harbor infectious microorganisms or even neoplastic cells.[1] The etiology of the pericardial effusion may vary from infectious (viral, bacterial, fungal, or parasitic), inflammatory or rheumatologist or immune-mediated (systemic lupus erythematosus, rheumatoid arthritis, and Sjogren syndrome), cancers (like Lung Cancer), following trauma of any kind, which might rupture the surrounding great vessels leading to the leakage of blood into the pericardial space, cardiac causes (after myocardial infarction, after a surgery to the heart, or due to rupture in the walls of the heart, congestive heart failure), vascular (type A aortic dissection), idiopathic, radiation-induced, renal pathologies (chronic kidney disease, renal failure), cirrhosis, myxedemic hypothyroidism, ovarian hyperstimulation syndrome, and drug-induced.[1] In nations with a high *Mycobacterium tuberculosis* load, tuberculosis (TB) stands out to be the leading cause of pericardial effusion[2–4] and 1–2% of patients with pulmonary TB and 1% of all autopsied cases of TB have been seen to develop tuberculous pericarditis followed by pericardial effusion.[5] In a 2018 Indian study conducted by Pradhan A et al., it was found that out of 55 patients of moderate to large pericardial effusion, a majority of 35 (63.64%), had its etiology attributing to TB.[6]

Tuberculosis (TB) is an infectious disease, caused by *Mycobacterium tuberculosis* bacteria. It has rankings in the world's top ten most mortality and morbidity-causing diseases and is also the most common single infectious disease cause of mortality worldwide. Almost 25% of the world's population is affected by the disease, and developing and underdeveloped countries are at far greater risk.[7] The lung is the target organ for TB (pulmonary TB), but other organs might also be affected, leading to what might be termed extrapulmonary tuberculosis (EPTB). Extrapulmonary TB leads to 10–20% of the manifestations seen in immunocompetent patients.[8] Tuberculous pericarditis has a high mortality rate of 17–40%, seen over six months.[9] Multidrug-resistant TB (MDR TB) is caused by *Mycobacterium tuberculosis* bacteria which are resistant to at least isoniazid and rifampin10, which makes it all the more difficult to treat, thus increasing the burden of the disease in society. The diagnostic criteria for tuberculous pericarditis in endemic TB countries,[11] are:

- Definite Tuberculous pericarditis:
  - Tubercle bacilli are found in stained smears or cultures of pericardial fluid; and/or,
  - Tubercle bacilli or caseating granuloma are found on histological examination of the pericardium.[11]
- Probable tuberculous pericarditis
  - Evidence of pericarditis in a patient with tuberculosis demonstrated elsewhere in the body; and/or,

- Lymphocytic pericardial exudate with elevated ADA activity; and/or,
- Good response to antituberculosis chemotherapy.[11]

The diagnostic approach of a suspected tuberculous pericarditis case, as proposed in a study by Mayos BMI et al., in the year 2005,[11] states that:

- Initial Evaluation:
  - Chest Radiograph
  - Echocardiogram: A large pericardial effusion along with frond-like projections, and thick "porridge-like" exudate is suggestive of an exudate but not specifically of a tuberculous etiology.[11]
  - Computerized Tomography (CT) scan, pericardial effusion, with thickening (>5 mm) and typical mediastinal and tracheobronchial lymphadenopathy (>10 mm, hypodense centers, matting), with sparing of hilar lymph nodes.[11]
  - Culture of the following samples:
    - Sputum; and/or
    - Gastric aspirate; and/or
    - Pericardial fluid
  - Lymph node biopsy (if pericardial fluid can't be accessed, but lymphadenopathy has been detected).[11]
- Pericardiocentesis
  - Therapeutic Pericardiocentesis: if cardiac tamponade is present.
  - Diagnostic Pericardiocentesis: in all suspected tuberculous pericarditis patients:
    - Direct inoculation of the pericardial fluid into double-strength liquid Kirchner culture medium, followed by culture for *Mycobacterium tuberculosis*.
    - Pericardial fluid protein and lactate dehydrogenase (LDH), and serum protein and LDH (Biochemical tests to distinguish between an exudate and a transudate).
    - Adenosine deaminase (ADA), interferon-gamma (IFN-γ), and lysozyme assays (indirect tests/markers for *Mycobacterium tuberculosis* infection).[11]
- Pericardial biopsy:
  - Therapeutic biopsy
  - Diagnostic biopsy (only in non-endemic regions).[11]

Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), or GeneXpert technology was a breakthrough advancement in the diagnosis of TB. The World Health Organization approved the CBNAAT in 2010 for rapid diagnosis of *Mycobacterium tuberculosis* bacterium, with the time taken to produce results being reduced to almost 2 h, and simultaneous detection of rifampicin resistance in the bacteria detected by it. This technique has been proposed and has been adopted as an initial diagnostic modality in countries with high TB load for faster detection of TB cases, along with their Rifampicin resistance pattern. However, to date, extremely few studies have analyzed the efficacy of CBNAAT in the diagnosis of tuberculous pericardial effusion.[12]

Adenosine Deaminase (ADA) enzyme, most commonly found in lymphocytes and myeloid cells, is involved in purine metabolism, which catabolizes adenosine. It's primarily engaged in the immune maintenance of the body.[13] ADA levels are found to rise in inflammatory effusions (exudative conditions), like pleural, pericardial, or joint effusion, whose etiology might range from bacterial infections, granulomatous conditions like TB, and sarcoidosis, cancers, and autoimmune conditions like lupus, vasculitis.[14,15] ADA in general undergoes an increment in neutrophil-predominant exudates. Hence, it is not of much diagnostic value in the background of neutrophil-predominant effusions.[16] But, when it comes to lymphocyte-predominant exudates, ADA is seen to rise exclusively in TB.[14,15]

2. Methodology

It was a cross-sectional observational study, conducted from January 2021 to December 2021, in the Department of Respiratory Medicine, in collaboration with the departments of Cardiology and Microbiology, in a North Indian Tertiary Health Care Centre. All patients with pericardial effusion, admitted via the Emergency unit, satisfying at least two of the following inclusion criteria, were included in our study:

- Pericardial fluid showing lymphocytosis on cytological examination
- Pericardial fluid ADA levels >40 U/mL
- CBNAAT positive for *Mycobacterium tuberculosis*
- Smear positive for acid-fast bacilli (AFB)
- Evidence of active tuberculosis anywhere else in the body.

The following group of patients were not included in our study:

- Minimal to Mild pericardial effusion
- Not satisfying the inclusion criteria
- Refusal to give consent
- Terminal illness with life expectancy <1 year
- Multiple Organ Dysfunction Syndrome
- Secondary MDR cases.

Data was collected from patients satisfying the inclusion criteria, and not meeting any of the exclusion criteria, after written informed consent from these patients, and they were well explained of the entire procedure. A detailed history and their demographic profile were recorded, to begin with. Vital clinical parameters, like Blood pressure, and Pulse rate, were recorded next. Following this, General and systemic examinations were performed. After this, the following laboratory investigations were performed: Complete blood count, blood urea, serum creatinine, Liver function test, viral markers (Hepatitis B and C, and Human Immunodeficiency Virus), chest X-ray, electrocardiography, Echocardiography, thyroid profile, computerized tomography of the chest, and blood antinuclear antibody (ANA) levels. A 2D echocardiogram was also performed. Under all aseptic precautions and Local anesthesia, pericardial fluid was drained via a subxiphoid route under echocardiographic guidance. Pericardial fluid was analyzed for color, cells, protein levels, malignant cells, sugar, Adenosine Deaminase (ADA), total leukocyte count, and differential leukocyte count. CBNAAT, Polymerase Chain Reaction (PCR) for *Mycobacterium tuberculosis*, gram staining, AFB

staining, and cultures and drug sensitivity testing were also performed on the pericardial fluid.

A convenient sampling method was used for the selection of patients for participation in our study. SPSS-v23 (IBM, USA) was used for the statistical calculation. The chi-square test, Fisher exact test, and Mann-Whitney *U* test were used to calculate the p-values as appropriate. $p < 0.05$ was considered to be statistically significant.

The study was approved by the Institutional Ethics Committee. All procedures were performed according to the ethical standards of human experimentation and the Helsinki Declaration (Rev. 2013).

3. Results

The total number of patients who were enrolled in our study was(n) 52. Their average age was 41.85 ± 17.88 years (The median age was 38 years, and the range was from 20 years to 90 years). The age group-wise distribution of the patients is shown in Fig. 1. 30 (57.7%) patients were males and the rest were females.

Of the 52 patients with pericardial effusion, 16 (30.77%) were positive for *Mycobacterium tuberculosis* bacilli on CBNAAT. Out of these 16, 2 (12.5%) were found to be Rifampicin resistant on CBNAAT. On culture, only 8 (15.38%) were positive for the *Mycobacterium tuberculosis* bacilli, out of which 6 (11.54%) were sensitive to first-line anti-tubercular drugs, whereas 2 (3.85%) (the same 2 which were Rifampicin resistant on CBNAAT) were resistant to both Isoniazid and Rifampicin (Fig. 2).

The number of patients with pericardial effusion with comorbidities like hypertension, diabetes mellitus, and obesity, were not found to have a significant difference among the groups detected positive in CBNAAT, and those detected negative in CBNAAT. Amongst the symptoms, only the number of patients presenting with cough was found to have a significant difference ($p = 0.02$) between the two groups, whereas, dyspnea and fever didn't have any such significant difference. Even, the number of patients positive for the Purified Protein Derivative (PPD) test, was not found to have any significant difference between the two groups (Table 1).

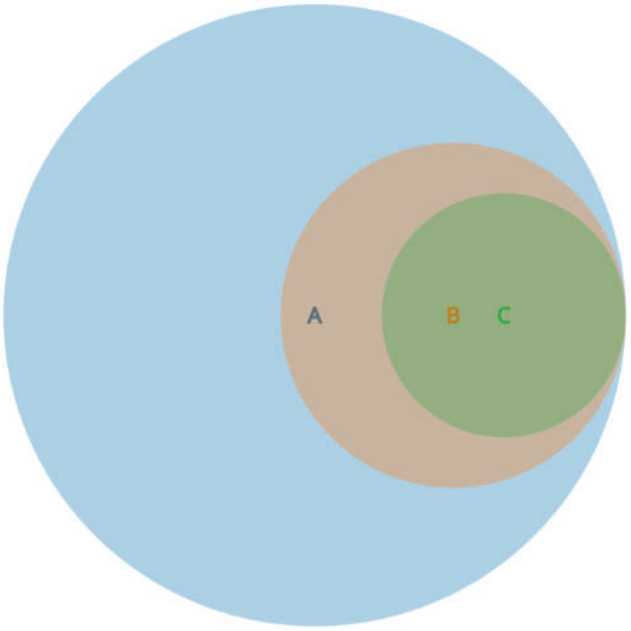


Fig. 2 – Venn Diagram showing patient distribution. (The Blue circle with A as its centre represents all patients with Pericardial effusion; the orange circle with B as its centre represents all patients who were positive on CBNAAT; and the green circle with C as its centre represents all patients who were positive on Culture.)

The summary of the test results of the various laboratory parameters in our patients has been summarized in Table 2. Only pericardial fluid ADA levels were found to have a significant difference between the patients detected positive and negative in CBNAAT respectively, whereas the Total Leukocyte Count, percentage of lymphocytes and neutrophils out of the total number of leukocytes, pericardial fluid sugar levels, and pericardial fluid protein levels, were found to have no significant difference between the two groups of patients (Table 2).

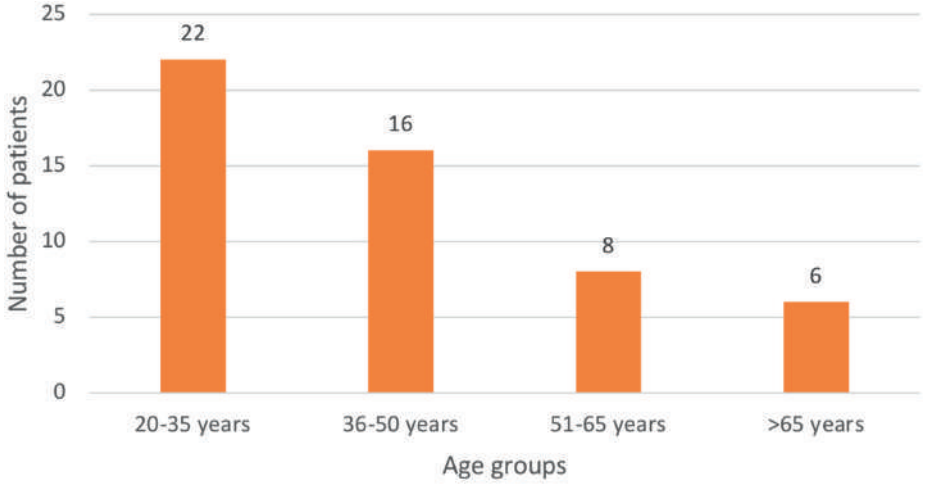


Fig. 1 – The age group-wise distribution of the patients.

Table 1 – Comparison of number of patients with different comorbidities, symptoms, and PPD Test results between CBNAAT positive and negative groups.

		CBNAAT					p-value χ2 test/Fisher exact test	
		M.TB DETECTED		M.TB NOT DETECTED		Total		
		N	%	N	%	N	%	
Hypertension	Yes	0	.0%	6	100.0%	6	100.0%	0.529
	No	16	34.8%	30	65.2%	46	100.0%	
	Total	16	30.8%	36	69.2%	52	100.0%	
Diabetes Mellitus	Yes	4	50.0%	4	50.0%	8	100.0%	0.563
	No	12	27.3%	32	72.7%	44	100.0%	
	Total	16	30.8%	36	69.2%	52	100.0%	
Obesity	Yes	6	60.0%	4	40.0%	10	100.0%	0.281
	No	10	23.8%	32	76.2%	42	100.0%	
	Total	16	30.8%	36	69.2%	52	100.0%	
Dyspnea	Yes	12	30.0%	28	70.0%	40	100.0%	1.000
	No	4	33.3%	8	66.7%	12	100.0%	
	Total	16	30.8%	36	69.2%	52	100.0%	
Cough	Yes	8	80.0%	2	20.0%	10	100.0%	**0.020***
	No	8	19.0%	34	81.0%	42	100.0%	
	Total	16	30.8%	36	69.2%	52	100.0%	
Fever	Yes	10	62.5%	6	37.5%	16	100.0%	0.060
	No	6	16.7%	30	83.3%	36	100.0%	
	Total	16	30.8%	36	69.2%	52	100.0%	
Tuberculin Sensitivity Test	Positive	6	60.0%	4	40.0%	10	100.0%	0.281
	Negative	10	23.8%	32	76.2%	42	100.0%	
	Total	16	30.8%	36	69.2%	52	100.0%	

A receiver operating characteristic (ROC) curve was plotted, with CBNAAT positive being the dependent variable and pericardial fluid ADA levels being the test variable (Fig. 3). The area under the curve (±SD) is 0.854 ± 0.078. p-value (non-parametric assumption) was 0.005 (significant). The lower bound and upper bound of the asymptotic 95% confidence interval were 0.701 and 1.008 respectively. Hence, the null hypothesis was rejected. The optimal cut-off value for ADA levels was found to be 37.9U/L, with a sensitivity of 1.0, and specificity of 0.5.

4. Discussion

Although CBNAAT provides rapid results, culture, which also includes drug-susceptibility testing of the growing bacteria, is considered the gold standard method for diagnosis of pulmonary TB.[17] But when it comes to tuberculous pericardial effusion, culture (sensitivity ranging from 53 to 75%[18]) is comparatively less sensitive as compared to CBNAAT (sensitivity of 63.8–78%, and specificity of 100%[19,20]). Moreover, the CBNAAT produces results within 2 h, as compared to almost 42 days by culture.[21] Pandie S et al., in 2014, were the first to scientifically analyze the diagnostic accuracy of the Xpert-MTB/RIF test in a large cohort (from Cape Town, South Africa), and they concluded that, when combined with either ADA or unstimulated IFNγ (uIFNγ), Xpert-MTB/RIF test can produce more than 97% sensitivity and specificity for diagnosis of tuberculous pericarditis.[19] Culture and/or pericardial histology by biopsy was considered the gold standard. 49% were classified as definite tuberculous pericarditis, 33% as probable tuberculous pericarditis, and 18% as non-tuberculous pericarditis. Xpert-MTB/RIF had a sensitivity of 63.8% and a specificity of 100%. Sharma et al. also analyzed the performance of GeneXpert in 1274 patients from India and claimed a high sensitivity and specificity of 71% and 95%, respectively, compared to culture.[22] In our study, only half the number of patients who were diagnosed via CBNAAT were

Table 2 – Comparison of various laboratory parameters between CBNAAT positive and negative groups.

	CBNAAT						p-value Mann Whitney *U* test
	M.TB DETECTED		M.TB NOT DETECTED		Total		
	Mean	SD	Mean	SD	Mean	SD	
TLC (/mm^3)	3540.00	3256.39	2114.17	2556.41	2552.88	2804.19	0.266
Lymphocytes (%)	81.25	15.29	78.50	16.73	79.35	16.05	0.633
Neutrophils (%)	18.75	15.29	21.50	16.73	20.65	16.05	0.633
SUGAR (mg/dL)	31.29	38.62	53.52	33.13	46.68	35.68	0.071
PROTEIN (g/dL)	5.19	.78	5.65	.64	5.51	.70	0.148
ADA (U/L)	85.91	37.60	39.78	24.31	53.97	35.63	**0.005***

Bold asterisk means p value <.05 and it is significant.

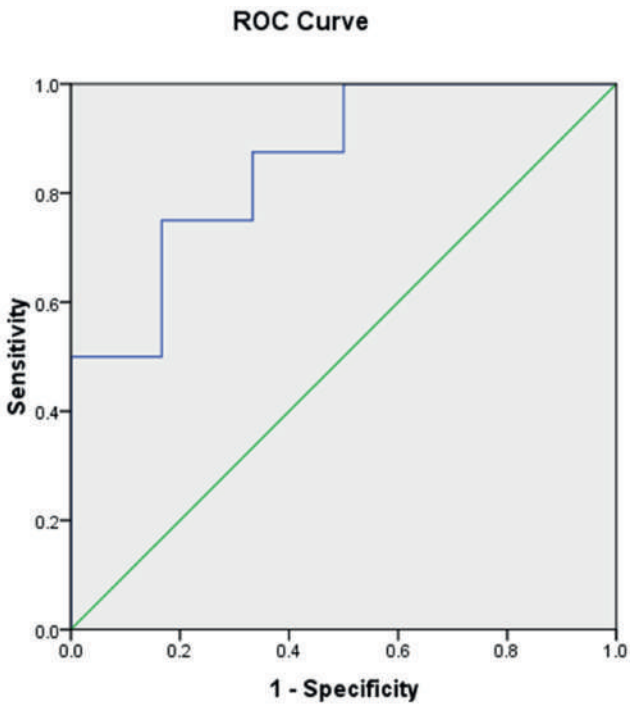


Fig. 3 – Receiver operating characteristic (ROC) curve, with CBNAAT positive as the dependent variable and pericardial fluid ADA levels as the test variable.

detected on culture to be *Mycobacterium tuberculosis* positive (30.77% (16) of total patients with pericardial effusion, on CBNAAT vs 15.38% (8), on culture). However, culture had successfully diagnosed the same two patients to be Rifampicin and isoniazid-resistant, who were detected as Rifampicin resistant on CBNAAT.

Chopra A. et al. observed the presence of hypertension in patients with pericardial effusion.[23] There is very little literature available on whether patients with tuberculous pericardial effusion have more prevalence of hypertension or not, as compared to patients with pericardial effusion due to other etiologies. In our study, we couldn't find any significant difference between the two groups ($p = 0.529$). There are not many studies reporting any significant correlation between the prevalence of diabetes mellitus and obesity in patients with pericardial effusion. In our study, no significant difference was found in their prevalence between patients with tuberculous pericardial effusion and pericardial effusion due to other causes ($p = 0.563$ and 0.281 respectively).

Tuberculous pericardial effusion has an insidious onset and presents with fever, night sweats, fatigue, loss in weight, chest pain, cough, and breathlessness.[24–27] Right hypochondriac pain due to liver congestion has been reported as well.[25,26,28] In our study, only the number of patients with cough as a presentation was seen to have a significant difference between the patients with pericardial effusion due to TB and those due to other etiologies ($p = 0.02$). There was no significant difference in the number of patients presenting with fever ($p = 0.06$) and dyspnea ($p = 1.00$), between the two groups.

Tuberculin skin test (TST) using purified protein derivative, is said to produce a positive skin reaction when an induration of ⩾10 mm is produced, and it is said to be a strongly positive response when induration is of ⩾15 mm, which may or may not involve skin excoriation. In a report by Rooney JJ et al., 100% of patients with tuberculous pericardial effusion had positive TST[29] and in another study by Strang JIG et al., 99.58% of patients had positive TST, with 78% having strongly positive TST.[30] Cherian G et al., have also reported an induration of 16.4 mm.[31] Strongly positive TST holds significant value when it is in a patient with tissue granuloma, but without acid-fast bacilli or when on CT scan, a typical non-hilar mediastinal adenopathy is seen.[32] However, in our study, there was no significant difference observed in the number of patients positive for TST between the group with patients with pericardial effusion due to TB and those due to other etiologies ($p = 0.281$).

TB, being a lymphocyte-predominant condition, expectantly the tuberculous pericardial effusion group had a higher average lymphocyte percentage and a lower neutrophil percentage, as compared to the control group, but the difference amongst the two groups was not significant (p for both = 0.633). Tuberculous pericardial effusion is an exudative condition leading to a rise in the protein levels in the pericardial fluid, and there is consumption of sugar by the *Mycobacterium tuberculosis* bacilli, leading to a decrease in its level in the pericardial fluid. However, these findings are not specific, and a similar picture can be seen in pericardial effusions due to other exudative and bacterial causes. In our study, average sugar levels were lower in the tuberculous group, but protein levels were higher in the control group. However, the differences between the two groups were again not significant (p for sugar levels = 0.071, and p for protein levels = 0.148).

In lymphocyte-rich effusions, the most commonly considered cut-off for ADA suggestive of TB is 40 U/L, which had been found to have a sensitivity of 87%–93% and specificity of 89%–97%.[15,33] But, if the cut-off is considered to be 35 U/L, sensitivity increases to 93%–95%, but on the other hand, specificity decreases to 74%–90%.[16,19,34] With a pretest probability of 70% for TB in a lymphocyte-rich pericardial effusion in patients from a country where TB is endemic, a pericardial fluid ADA level of≥40 U/L results in a post-test probability of 96%, while levels≤40 U/L leads to a post-test probability of 19%.[35,36] In our study, pericardial fluid ADA levels were found to be significantly higher ($p = 0.005$) in the group with patients of tuberculous pericardial effusion, as compared to the other group with non-tuberculous pericardial effusion patients. The cut-off value for pericardial fluid ADA levels for detection of tuberculous pericardial effusion was found to be 37.9U/L, with a sensitivity of 1.0, and specificity of 0.5. In the study by Pandie S et al., ADA with a cut-off of 35 IU/L (sensitivity of 95.7%) and uIFNγ with a cut-off of 44 pg/ml (sensitivity of 98.5%), and a negative likelihood ratio of 0.05 (0.02–0.10), were found to be significant markers in tuberculous pericardial effusion. However, the specificity and positive likelihood ratio of uIFNγ was more than that of ADA. Whereas, the sensitivity and negative predictive value of both the above markers were more than that of CBNAAT.[19] A 1999 study by Dogan R et al., reported the cut-off value for pericardial fluid ADA levels to be 50IU/L, with a sensitivity of 1 and specificity of 0.83. No correlation was found

between ADA activity in the serum and the pericardial fluid.[37] In a systematic review of 222 cases by Tuon FF et al., including studies by Lee et al. (2002, Korea),[38] Koh et al. (1997, Korea),[39] Reuter et al. (2005, South Africa),[40] Dogan et al. (1999, Turkey),[37] and Martinez-Vazquez et al. (1986, Spain),[41] it was found that measuring ADA levels as a marker of tuberculous pericarditis had sensitivity 88% and a specificity of 83%, with a positive predictive value of 83%, and a negative predictive value of 88%. The cut-off was found to be 40U/L, with a positive likelihood ratio of 5.3, and a negative likelihood ratio of 0.14. The area under the SRIC curve was 0.9539, which confirms the high sensitivity and specificity of pericardial fluid ADA levels as a diagnostic tool for tuberculous pericardial effusion.[42]

5. Conclusion

Culture is the gold standard method to diagnose tuberculosis. Detection of MTB on pericardial fluid culture is very uncommon, though in our study, culture came out positive in 16% of patients, and 4% were resistant to rifampicin and isoniazid. Higher ADA levels in pericardial fluid are an indicator of tuberculous pericardial effusion. Early diagnosis of drug-resistant tubercular pericardial effusion is a cornerstone of effective treatment. The present study highlights the utility of Adenosine deaminase, CBNAAT, and culture tests to detect drug-resistant pericardial effusion.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Willner DA, Goyal A, Grigorova Y, et al. Pericardial Effusion [Internet]. StatPearls; [cited 2023 Apr16]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK431089/#_NBK431089_pubdet_.
2. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors. This statement was endorsed by the council of the infectious disease society of America, september 1999. *Am J Respir Crit Care Med 2000*. July 1999;161:1376.
3. Larrieu AJ, Tyers GF, Williams EH, Derrick JR. Recent experience with tuberculous pericarditis. *Ann Thorac Surg*. 1980;29:464.
4. Mayosi BM, Volmink JA, Commerford PJ. Pericardial disease: an evidence-based approach to diagnosis and treatment. In: Yusuf S, Cairns JA, Camm AJ, Fallen BJ, eds. *Evidence-Based Cardiology*. second ed. London: BMJ Books; 2003:735–748.
5. Fowler NO. Tuberculous pericarditis. *JAMA*. 1991;266:99–103.
6. Pradhan A, Vishwakarma P, Bhandari M, et al. Demographic, clinical and etiological profile of pericardial effusion in India: a single centre experience. *IJTB*. 2022;69(2):220–226.
7. World Health Organization. Global tuberculosis report. https://www.aidsdatahub.org/resource/global-tuberculosis-report-2019; 2019.
8. Natarajan A, Beena PM, Devnikar AV, Mali S. A systemic review on tuberculosis. *IJTB*. 2020;67:295–311. https://doi.org/10.1016/j.ijtb.2020.02.005 [PMID: 32825856 DOI:.
9. Isiguzo G, Du Bruyn E, Howlett P, Ntsekhe M. Diagnosis and management of tuberculous pericarditis: what is new? *Curr Cardiol Rep*. 2020;22(2). https://doi.org/10.1007/s11886-020-1254-1. PMID: 31940097.
10. https://www.cdc.gov/tb/topic/drtb/default.htm#:~:text=Multidrug%2Dresistant%20TB%20(MDR%20TB)%20is%20caused%20by%20TB,the%20treatment%20of%20MDR%20TB.
11. Mayos BMI, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation*. 2005;112:3608–3616. https://doi.org/10.1161/CIRCULATIONAHA.105.543066.
12. Negi N, Das BK. Genexpert technology: a new ray of hope for the diagnosis of tuberculour pericardial effusion. *J Pract Cardiovasc Sci*. 2015;1:233–240.
13. Wilson DK, Rudolph FB, Quiocho FA. Atomic structure of adenosine deaminase complexed with a transition-state analog: understanding catalysis and immunodeficiency mutations. Bibcode:1991Sci...252.1278W *Science*. May 1991;252(5010):1278–1284. https://doi.org/10.1126/science.1925539. PMID 1925539.
14. Arroyo M, Soberman JE. Adenosine deaminase in the diagnosis of tuberculous pericardial effusion. *Am J Med Sci*. 2008;335(3):227–229. https://doi.org/10.1097/MAJ.0b013e3180cab71a.
15. Reuter H, Burgess L, van Vuuren W, Doubell A. Diagnosing tuberculous pericarditis. *QJM*. 2006;99(12):827–839. https://doi.org/10.1093/qjmed/hcl123.
16. Porcel JM, Esquerda A, Bielsa S. Diagnostic performance of adenosine deaminase activity in pleural fluid: a single-center experience with over 2100 consecutive patients. *Eur J Intern Med*. 2010;21(5):419–423. https://doi.org/10.1016/j.ejim.2010.03.011.
17. https://www.cdc.gov/tb/education/corecurr/pdf/chapter4.pdf.
18. Kyriakakis CG, Mayosi BM, de Vries E, Isaacs A, Doubell AF. An approach to the patient with suspected pericardial disease. *SAMJ (S Afr Med J): S Afr Med J*. 2016;106(2):151–155. https://doi.org/10.7196/SAMJ.2016.v106i2.10328.
19. Pandie S, Peter JG, Kerbelker ZS, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon-γ in a high burden setting: a prospective study. *BMC Med*. 2014;12:101.
20. Yu G, Ye B, Chen D, et al. Comparison between the diagnostic validities of Xpert MTB/RIF and interferon-gamma release assays for tuberculous pericarditis using pericardial tissue. *PLoS One*. 2017 Dec 6;12(12):e0188704. https://doi.org/10.1371/journal.pone.0188704.
21. Isiguzo G, Du Bruyn E, Howlett P, Ntsekhe M. Diagnosis and management of tuberculous pericarditis: what is new? *Curr Cardiol Rep*. 2020 Jan 15;22(1):2. https://doi.org/10.1007/s11886-020-1254-1. PMID: 31940097; PMCID: PMC7222865.
22. Sharma SK, Kohli M, Chaubey J, et al. Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India. *Eur Respir J*. 2014;44:1090–1093.
23. Chopra A, Highland KB, Kilb E, Huggins JT. The relationship of pleural and pericardial effusion with pulmonary hemodynamics in patients with pulmonary hypertension. *Am J Med Sci*. 2021;361:731–735. https://doi.org/10.1016/j.amjms.2021.01.003.
24. Schrire V. Experience with pericarditis at Groote Schuur Hospital, Cape Town: an analysis of one hundred and sixty cases over a six-year period. *S Afr Med J*. 1959;33:810–817.

25. Hageman JH, d'Esopo ND, Glenn WWL. Tuberculosis of the pericardium: a long-term analysis of forty-four proved cases. *N Engl J Med*. 1964;270:327–332.
26. Desai HN. Tuberculous pericarditis: a review of 100 cases. *S Afr Med J*. 1979;55:877–880.
27. Fowler NO, Manitas GT. Infectious pericarditis. *Prog Cardiovasc Dis*. 1973;16:323.
28. Strang JIG. Tuberculous pericarditis in transkei. *Clin Cardiol*. 1984;7:667–670.
29. Rooney JJ, Crocco JA, Lyons HA. Tuberculous pericarditis. *Ann Intern Med*. 1970;72:73–78.
30. Strang JIG, Kakaza HHS, Gibson DG, et al. Controlled clinical trial of complete open surgical drainage and of prednisolone treatment of tuberculous pericardial effusion in the Transkei. *Lancet*. 1988;ii:759–764.
31. Cherian G, Habashy AG, Uthaman B, et al. Detection and follow-up of mediastinal lymph node enlargement in tuberculous effusions using computed tomography. *Am J Med*. 2003;114:319–322.
32. Cherian G. Diagnosis of tuberculous aetiology in pericardial effusions Postgraduate. *Med J*. 2004;80:262–266.
33. Koh KK, Kim EJ, Cho CH, et al. Adenosine deaminase and carcinoembryonic antigen in pericardial effusion diagnosis, especially in suspected tuberculous pericarditis. *Circulation*. 1994;89(6):2728–2735. https://doi.org/10.1161/01.CIR.89.6.2728.
34. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation*. 2005;112(23):3608–3616. https://doi.org/10.1161/CIRCULATIONAHA.105.543066.
35. Syed FF, Mayosi BM. A modern approach to tuberculous pericarditis. *Prog Cardiovasc Dis*. 2007;50(3):218–236. https://doi.org/10.1016/j.pcad.2007.03.002.
36. Chau E, Sarkarati M, Spellberg B. Adenosine deaminase diagnostic testing in pericardial fluid. *JAMA*. 2019 Jul 9;322(2):163–164. https://doi.org/10.1001/jama.2019.7535. PMID: 31199430; PMCID: PMC6697089.
37. Dogan R, Demircin M, Sarigül A, Ciliv G, Bozer AY. Diagnostic value of adenosine deaminase activity in pericardial fluids. *J Cardiovasc Surg*. 1999 Aug;40(4):501–504. PMID: 10532206.
38. Lee JH, Lee CW, Lee S, et al. Comparison of polymerase chain reaction with adenosine deaminase activity in pericardial fluid for the diagnosis of tuberculous pericarditis. *Am J Med*. 2002;15:519–521.
39. Koh KK, In HH, Lee KH, et al. New scoring system using tumor markers in diagnosing patients with moderate pericardial effusions. *Int J Cardiol*. 1997;61:5–13.
40. Reuter H, Burgess LJ, Doubell AF. The role of chest radiography in diagnosing patients with tuberculous pericarditis. *Cardiovasc J South Afr*. 2005;16:108–111.
41. Martinez-Vazquez JM, Ribera E, Ocana I, Segura RM, Serrat R, Sagrista J. Adenosine deaminase activity in tuberculous pericarditis. *Thorax*. 1986;41:888–889.
42. Tuon FF, Litvoc MN, Lopes MI. Adenosine deaminase and tuberculous pericarditis–a systematic review with meta-analysis. *Acta Trop*. 2006 Aug;99(1):67–74. https://doi.org/10.1016/j.actatropica.2006.07.004.

Indian Journal of Tuberculosis 71 (2024) S67–S71

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Treatment outcomes of multidrug resistant tuberculosis (MDR TB) treated with bedaquiline under programmatic management of drug resistant tuberculosis at a tertiary care hospital in Punjab, India

Dr Sudesh Kumari [a], Dr Aditi Gupta [b,*], Dr Vishal Chopra [a], Dr Snehinder Kaur [c]

[a] Pulmonary Medicine, GMC, Patiala, India
[b] Pulmonary Medicine, BRAIMS, Mohali, India
[c] NDRTBC, GMC, Patiala, India

ARTICLE INFO

Keywords:
Bedaquiline
MDR TB
Multi drug resistant tuberculosis
PMDT
Treatment outcome
Bdq
adverse events

ABSTRACT

Introduction: MDR TB is a serious global concern which is hampering TB elimination goals badly. Standardized MDR TB regimen had high default rates, more side effects and poor treatment outcomes. Bedaquiline is a newer anti-tubercular drug which has made oral regimens possible for MDR TB. We aimed to study the outcomes of MDR TB patients treated with Bdq containing regimens.

Methods: 155 patients of MDR TB on Bdq containing regimen enrolled at GMC, Patiala under NTEP from 2017 to 2020 were enrolled retrospectively.

Results: Out of 155 patients enrolled, 82 (52.9 %) were cured, 31 (20 %) completed treatment, 18 (11.6 %) defaulted, 22 (14.2 %) died and 2 (0.12 %) failed treatment.

Conclusion: Bdq is well tolerated with very less side effects and has better outcomes as compared to standard MDR regimens which were followed earlier.

1. Introduction

Tuberculosis (TB) was declared as a global emergency by WHO in 1993[1] and despite all efforts since then, we are unable to eliminate this communicable preventable disease. WHO targets to end TB by 2035 by 95 % reduction in TB deaths, 90 % reduction in TB incidence rates and zero catastrophic costs due to TB. Nevertheless, 10.6 million people were diagnosed with TB in 2021 globally and out of these around 4.5 lacs had MDR TB which was 3.6 % among new cases and 18 % among previously treated as per WHO Global report 2022.[2]

Multi drug resistant TB (MDR TB) is a serious global concern which requires urgent prompt attention at all the levels of healthcare. Standard treatment for MDR TB has significant adverse effects, more default rates and less cure rates as compared to drug sensitive TB which highlights the need of new anti TB drugs that are more effective and better tolerated.[3] First anti TB drug, Streptomycin was discovered in 1944 followed by Rifampicin(R) in 1960,[4] and for around 40 years there was no new drug approved for TB treatment. In 2012, Bedaquiline (Bdq) got FDA approval for treating MDR TB for above 18 years only as a part of combination therapy. Bdq, a diarylquinoline (TMC207) targets mycobacterial ATP synthase complex where it binds at a defined binding site and the stereogenic center configuration of this drug plays an important role in its activity. It is well absorbed orally with max plasma concentration, tmax at 5 h post dose. It follows a triphasic elimination and has a long terminal half life of 173 hr[5] The early bactericidal activity of Bdq is comparable to Isoniazid(H) and R. However, no bactericidal action occurs during the first 2–4 days of therapy and after blocking ATP synthesis, it takes at least 3–4 days from depletion of ATP to disruption of intracellular pH homeostasis finally leading to its bactericidal activity.[6] We aim to analyse the tolerability and safety of Bdq for MDR TB treatment under National TB elimination Programme (NTEP).

2. Material and methods

2.1. Study design and setting

This retrospective observational study was conducted at TB hospital, Pulmonary medicine department at Government medical college,

* Corresponding author. Pulmonary Medicine, BRAIMS, Mohali, India.
E-mail addresses: sudeshkb@yahoo.co.in (D.S. Kumari), doc.aditigupta@gmail.com (D.A. Gupta), drvishalchopra@gmail.com (D.V. Chopra), Snehinderkaur61@gmail.com (D.S. Kaur).

https://doi.org/10.1016/j.ijtb.2023.12.003
Received 7 October 2023; Received in revised form 3 November 2023; Accepted 21 December 2023
Available online 27 December 2023

Patiala, India which serves 8 districts for MDR TB treatment in the state of Punjab namely Sangroor, Mansa, Malerkotla, SAS nagar, Fatehgarh sahib, Barnala, Patiala and Roopnagar. Patients from the above districts were referred to our hospital for diagnosis, management and further follow up. All sputum samples were processed at in house Intermediate Reference Laboratory (IRL). Patients were diagnosed as MDR TB on the basis of genotypic tests CBNAAT/gene expert or line probe assay (LPA) after sputum microscopy where CBNAAT confirms resistance to R and first line LPA confirms resistance of R and H both. It was followed by second line LPA for Levofloxacin, Moxifloxacin, Kanamycin and Capreomycin resistance forall the patients. Liquid culture was performed for all the patients under NTEP. Ethics approval (Trg.9 (310)2023/14808) was obtained from Government medical college, Patiala ethical committee.

2.2. Study population

Patients above 18 years of age with sputum AFB positive Pulmonary Koch's and resistance to at least R on CBNAAT were included in the study. MDR TB patients who were started on Bdq containing regimen from 2017 to 2020 were enrolled in the study as per PMDT guidelines 2017.[7] During the initial recruitment, Bdq was added under newer drug containing regimen (regimen 1) but later from October 2019, all oral longer regimen (regimen 2) was implemented. For regimen 1 Bdq was indicated in adult MDR-TB patients not eligible for the WHO-recommended shorter regimen which included MDR/RR-TB patients with resistance to any/all FQ OR to any/all SLI; XDR-TB patients; mixed pattern resistant TB patients; treatment failures of MDR-TB + FQ/SLI resistance OR XDR-TB; and MDR/RR-TB patients with extensive pulmonary lesions, advanced disease and others deemed at higher baseline risk for poor outcomes.Regimen 2 was indicated in all MDR TB patients whom shorter regimen was contraindicated as per PMDT 2019.[8]

Contraindications to Bdq were uncontrolled cardiac arrhythmia that required medication or having any of the following QT/QTc interval characteristics at screening: marked prolongation of QT/QTc interval, e.g. repeated demonstration of QTcF (Fredericia correction) interval >450 ms; and history of additional risk factors for Torsade de Pointes, e.g. heart failure, hypokalaemia, family history of long QT syndrome.

2.3. Pre-treatment evaluation

During the initial recruitment period in 2017, patients were admitted for 14 days for initiating Bdq under newer drug containing regimen (regimen 1) but later since the implementation of all oral longer regimen (regimen 2) from October 2019, treatment was offered on outpatient basis. Blood investigations included complete blood counts, liver function tests, renal function tests, serum calcium, magnesium and lipase, urine pregnancy test for female patients. Fundus examination was done for every patient. ECG with 12 leads and long lead was done. Daily QTc was calculated for 14 days for regimen 1 patients only. Hypocalcemia, hypomagnesaemia and hypokalemia were corrected before initiating Bdq. DRTB Expert committee which comprised of a pulmonologist, microbiologist, pharmacologist, medicine specialist, ENT, gynaecologist and psychiatrist took all the decisions regarding treatment initiation and modifications if required.

2.4. Treatment regimens

The type of regimen was decided on the basis of time of treatment initiation. For those whose treatment was initiated before October 2019 were put on regimen 1 and rest all on regimen 2.

Regimen 1 (as per PMDT 2017)- (6–9) Bdq (6) Kanamycin, Ethionamide, Cycloserine, Linezolid, Pyrazinamide, Clofazimine; (18) Ethionamide, Cycloserine, Linezolid, Clofazimine.

Regimen 2 (as per PMDT 2019)- (18–20) Bdq (6) Levoflox, Linezolid, Clofazimine, Cycloserine.

All patients received Tab. Bdq 400 mg once daily for the first 2 weeks and 200 mg 3 times a week (with at least 48 hr between doses) for the following 22 weeks, in combination with the background regimen as detailed above.

2.5. Follow up

Follow up was scheduled monthly till IP. At every FU visit, weight, AFB smear, sputum culture (from 3rd month onwards), creatinine, CBC, ECG were done till IP and quarterly in CP. Electrolytes (Na K Cl), Mg, Ca, Amylase, Protein, Lipase were done quarterly in IP. CXR, TSH and LFT were done at the end of IP and end of treatment. SL LPA was done if culture positive at the end of IP and/or extended IP or any time in CP, extended DST if any resistance on SL LPA. Any test could be performed in between if clinically indicated. Long term follow up was done at 6, 12, 18 and 24 months after treatment completion. All patients were monitored for ADRs in every follow up visit passively. Outcomes were defined as per PMDT 2017. Death, failure and lost to follow up were counted as unfavourable outcome whereas cured and treatment completed were considered successful outcomes.

2.6. Operational definitions

1. Culture conversion: when two consecutive cultures, taken at least 30 days apart, were found to be negative.
2. Culture reversion: when, after an initial culture conversion, two consecutive cultures, taken at least 30 days apart, were found to be positive.
3. Cure: Treatment completed without evidence of failure and three or more consecutive cultures taken at least 30 days apart during CP were negative including culture at the end of treatment.
4. Treatment completed: Treatment completed without evidence of failure but no record that three or more consecutive cultures taken at least 30 days apart were negative after the intensive phase.
5. Treatment failed: Treatment terminated or need for permanent regimen change of at least two or more anti-TB drugs in CP because of lack of microbiological conversion by the end of the extended intensive phase or microbiological reversion in the continuation phase after conversion to negative or evidence of additional acquired resistance to FQ or SLI drugs or adverse drug reactions (ADR).
6. Died: A patient who died for any reason during the course of treatment.
7. Lost to follow-up: A patient whose treatment was interrupted for one month or more for any reasons prior to being declared as failed.
8. Serious adverse event (SAE) was defined as any event which is life threatening or causing permanent disability or requiring prolonged hospitalization.

2.7. Data management and statistical analysis

Patient records were maintained at hospital level and district level under NTEP. Data was retrieved from PMDT register, treatment card, clinical inpatient file which was tabulated in excel spread sheets. All follow up visits and investigations were recorded manually. As many of the patients were on long term follow up, all the data was periodically checked and completed before the final analysis was carried out. Statistical analysis was done using SPSS software version 21.0. P value of less than 0.05 was considered significant statistically. Percentages, mean and median were calculated and chi square test was used to compare categorical variables and independent *t*-test was used to compare continuous variables. Association of Sex, malnutrition, previous history of ATT, drug resistance pattern, Diabetes and adverse events were compared for any association with the outcome.

Table 1
Baseline demographic characteristics of patients on Bdq containing regimen (n = 155).

	Characteristics	**Total n (%)**	Unfavourable outcome[a]	P value
1.	Gender			
	Male	86 (55.5)	11/78	0.164
	Female	69 (44.5)	12/60	
2.	Age (years, median/IQR)	38.62		
3.	Residence			
	Rural	65 (41.9)		0.755
	Urban	90 (58)		
4.	BMI,kg/m2 (mean ± SD)	18 ± 3.8		
	Malnutrition (<18.5 or >24.9)	95 (61.3)	16/81	0.087
5.	History of ATT			0.588
	New cases	31 (20)		
	First line ATT	117 (75.5)	16/102	0.574
	Second line ATT	104 (67)		0.328
	Injectable use	84 (54.2)	12/73	0.616
6.	Comorbidity			0.516
	Diabetes	27 (17.4)	3/24	
	COPD	1 (0.64)		
	Hepatitis C	2 (1.3)		
	Hypertension	3 (1.9)		
	Hypothyroidism	5 (3.22)		
	CAD	2 (1.3)		
	seizure	2 (1.3)		
	HIV	5 (3.22)		
7.	CXR			
	Unilateral Multi lobar	37 (23.9)	1/35	0.058
	Bilateral	85 (54.8)	20/74	
	Cavity	16 (10.3)	1/16	
	Bilateral cavity	11 (7)	0/9	
	WNL	6 (3.9)	2/5	

[a] Lost to follow up are excluded from the total. (eg.- out of 86 males 8 were lost to follow up.)

3. Results

3.1. Patient characteristics

In this study, 155 patients were enrolled (Table 1) where 86 (55.5 %) were males and 69 (45.5 %) were females. Mean age group enrolled was 35.08 years in patients with successful outcome and 42.16 years in unsuccessful. Majority of the patients were from urban area (90, 58 %) whereas rural were slightly less (65,41.9 %). Average weight and height of patients were 47.4 ± 10.76 kg and 162.17 ± 7.2 cm respectively. 95 (61.3 %) patients were malnourished with average BMI of 18.33 ± 3.99 kg/m^2. Diabetes (27,17.4 %) was the most common comorbidity followed by HIV, hypothyroidism, hypertension and Hepatitis B in the given order.

3.2. ATT history

Majority of the patients had previous history of ATT except 31 who were not treated for TB in the past. 117 had received first line ATT in the past whereas 104 received second line ATT as well and out of them 96 had history of injectables use.

3.3. Extent of CXR involvement

Majority had bilateral disease (85) followed by multilobar unilateral (37) and surprisingly 6 had normal CXR. CECT chest was not done in these patients. Possibility of small infiltrations which are not visible on CXR cannot be ruled out.

Table 2
Summary of adverse events with Bdq containing ATT in both the groups.

Adverse drug event	Group 1 (n = 101)	Group 2 (n = 54)	Total	Unfavourable outcome	P value
Nausea vomiting	19	17	36	6/34	
Deranged LFT	5	3	8	1/8	
QT prolongation	4	3	7	3/5	
Neuropathy	5	13	18	2/17	0.094
Arthralgia	7	11	18	1/17	0.025
Hearing loss	3	0	3	1/3	0.338
Psychosis	3	2	5	0/5	0.323
Suicidal tendencies	4	0	4	3/3	0.065
Depression	10	3	13	6/11	0.191
Blurring	6	2	8	2/7	1
Others	11	16			

Table 3
Treatment outcome in patients on Bdq containing regimen (n = 155).

	Regimen 1 (n = 101)	Regimen 2 (n = 54)	Combined
Cured	51 (50.5 %)	31 (57.4 %)	82 (52.9 %)
Treatment complete	22 (21.8 %)	9 (16.6 %)	31 (20 %)
Lost to follow up	13 (12.9 %)	5 (9.2 %)	18 (11.6 %)
Died	14 (13.8 %)	8 (14.8 %)	22 (14.2 %)
Failure	1 (0.9 %)	1 (1.85 %)	2 (0.12 %)

3.4. Drug resistance pattern

All 155 patients had sputum CBNAAT positive for R resistance. At baseline, 99 patients were resistant to Levofloxacin whereas 35 for both Levofloxacin and Moxifloxacin. Levofloxacin resistant is high as Bdq was started only in those who had FQ resistance in gp1. Hence, eligible patients enrolled in gp1 already had FQ resistance. Among SLI resistance pattern, 8 were resistant to both kanamycin and capreomycin whereas 4 for kanamycin alone. Kat G mutation was detected in 31 and inh A in only 1 whereas both together in 6 patients.

3.5. Adverse drug reactions

Nausea, vomiting ((36, 23.2 %) was the most common side effect followed by arthralgia (18,11.6 %) and neuropathy (18,11.6 %) (Table 2). Depression was diagnosed in 13 (8.3 %) patients where 4 (2.5 %) had suicidal tendencies and 5 (3.2 %) developed psychosis. 3 (1.9 %) developed hearing loss and 8 (5.16 %) had blurring of vision. Out of 5 patients who developed psychosis, 2 committed suicide. There were total 45 (29 %) serious adverse events reported in 155 patients. Suicidal tendencies and depression were associated with poor outcome independently.

3.6. Culture conversion

Under national programme, first culture was done at 3 months and only 15 patients (9.7 %) were culture positive at 3 months and only 2 remained positive at the end of 6 months. Hence, culture conversion at 3 months was markedly improved with addition of Bdq.

3.7. Treatment outcome

Out of 155 patient enrolled, there were 22 (14 %) deaths and 18 (11.6 %) were lost to follow up and 2 failures (1.2 %)were reported as shown in Table 3. Default or lost to follow up owing to severe ADR were high as ADR reporting was passive under the programme during the study period.

Table 4
Drug resistance pattern at the baseline and association with the outcome.

S No.	Drug	Resistance pattern	n (%)	Unfavourable outcome	P value
2.	SLI	Only k	4 (2.6)	1/3	0.194
		Both k + cp	8 (5.2)	0/8	
3.	Isoniazid	Kat G	31 (20)	4/29	0.257
		Both Inh A + Kat G	6 (3.9)	2/5	
		Only inh A	1 (0.6)	0/1	

4. Discussion

MDR TB is a major threat to TB elimination goals where early diagnosis and treatment is the key to break the chain of transmission. As per national DST survey 2016, drug resistance to H was 11.06 % of any resistance in new patients and 25.09 % in previously treated followed by Streptomycin (6.88,13.26), R (2.84,11.67), Levofloxacin (2.71,3.75), Capreomycin (1.04,0.85). Prevalence of resistance of FQ is not relevant in this study as FQ resistance was the eligibility criteria for Bdq containing regimen before 2019. 63.9 % of patients were resistant to levofloxacin and 22 % were resistant to both levofloxacin and moxifloxacin. Resistance to H was more as compared to SLI as observed in national DST survey 2016 [9] (Table 4).

Even after the diagnosis of MDR TB, treatment outcomes are not at all impressive. Drug intolerance, long duration, serious adverse events, high default rates lead to poor outcomes despite availability of investigations and free treatment across the country. Before the inclusion of Bdq, Standard MDR treatment for 24–27 months had favourable outcome in 33–68 % patients as shown in Table 5. Kanamycin containing regimens had very high rates of ototoxicity and nephrotoxicity adding to increase in default rates wherein 50.9 % had successful outcome when treated with shorter injectable based regimen.[10] Bdq has revolutionised the DRTB treatment where all oral regimens are possible now and these regimen are better tolerated, more effective and has improved adherence and outcomes.

MDR treatment is less effective and has more side effects as compared drug sensitive TB. Majority of the ADR in the study were managed with symptomatic treatment whereas 17 (11 %) patients required discontinuation of the offending drug in this study which is very less as compared to various other studies as it was a retrospective study and ADR monitoring was passive under programme in the study period leading to underreporting of mild ADR.[7,8,22,25] Here, Cycloserine was stopped in 8 (5.1 %) due to psychosis or depression or suicidal ideation, Linezolid in 10 (6.4 %) for peripheral neuropathy or blurring of vision, Kanamycin injection in 2 (1.2 %) due to hearing loss, Bdq in 4 (2.5 %) for QT prolongation and Ethionamide in 1 (0.6 %)for hypothyroidism. One patient died of sudden cardiac arrest at home which could be related to QT prolongation caused by Bdq or Levofloxacin. Total 8 (5.1 %) developed QT prolongation where only 4 required permanent discontinuation of Bdq and rest were managed with electrolytes correction.

Multiple systematic reviews have concluded that end treatment outcome with Bdq containing regimens are better with early culture conversion, lesser mortality and failure rates.[26,27] Various new regimens with Bdq are under trial , for instance Bpal regimen for R resistant TB comprising of 6 months of 4 oral drug therapy has revolutionised MDR TB treatment with 0% failure and 3% relapse among 70 patients whereas linezolid dose required modifications in 62% patients.[28] Emerging resistance and lack of availability of DST for Bdq are big hurdles which need to be addressed urgently before making Bdq as a part MDR TB treatment. Delayed diagnosis affects long term outcome in multiple ways. Up to 90 % MDR TB patients develop Post TB lung disease (PTLD) due to delayed diagnosis and longer less effective treatment requiring pulmonary rehabilitation.[29]

5. Limitations

Patients enrolled in this study were managed as per PMDT guidelines under ministry of health and family welfare. First follow up culture was not done before 3 months of treatment under programme. Hence, exact culture conversion cannot be commented upon. Causality of adverse event could not be assessed due to simultaneous use of various drugs except few specific side effects. Retrospective nature of the study and passive ADR reporting lead to underreporting of mild ADR as patients reported mainly moderate to severe ADR. Thirdly, two different regimens including Bdq were offered to the patients under the programme. Lastly, studies with larger number of patients are required for better results.

6. Conclusion

Bdq containing regimens are safer with better outcomes as compared to regimens containing injectables. DST for Bdq should be available at local level considering its rampant use in MDR TB. More translational research is required to overcome the huge dearth of oral drugs for MDR/XDR TB in the world of emerging resistance. Newer anti TB drugs are need of the hour to meet the goals of TB elimination by 2030.

Table 5
Comparison of outcomes of MDR TB in few recent studies conducted in India.

S no.	Author (Year of publication)	City	n	Treatment duration (months)	Cured (%)	Treatment completed (%)	Death (%)	Default (%)	Failure (%)
1.	Kalpesh Jain[11](2014)	Gujarat	130	24–27	39 %	5	23	19	13
2.	ZF Udwadia[12] (2014)	Mumbai	78	Around 22	68 %		1	16	15
3.	Arun K Yadav[13](2016)	Jaipur	115	24–27	63.4		11.3	15.6	
4.	Sangita V Patel[14](2016)	Vadodara	145	24	33.1	5.5	29.7	21.10	
5.	Neeta P N[15](2016)	Ballari, Karnataka	43	24	44.2		20.9	28	2.3
6.	SS Dole[16] (2017)	Solapur	146	24–27	58		14	19	9
7.	SL Suryawanshi[17] (2017)	Maharashtra	4024	24	29		21	19	
8.	AK Janmeja[18] (2017)	Chandigarh	140	24	55	7.9	16.4	9.3	3.6
9.	MA Waghmare[19] (2018)	Mumbai	194	24	35	13.4	20.15	11.8	11.3
10.	MM Parmar[20] (2018)	New Delhi	2264	24	34.5		28.4	29.6	7.5
11.	Nitin Gupta[21] (2018)	New Delhi	819	24	52	3	16	24	1
12.	AbhijitSingh[22] (2019)	Chennai	98		72.4 combined		10.2	7.1	10.2
13.	RP Takhar[23] (2019)	Kota	2860	24	35.66	9.09	25.87	26.57	2.44
14.	Nandini Sharma[24] (2020)	New Delhi	2690		41.18	9.77	17.9	20.55	3.38
15.	Mrinalini Das[25](2021)	Mumbai	70	24 Bdq + dlm	54	16	19	4	7

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Who Global Tuberculosis Programme. *TB : A Global Emergency, WHO Report on the TB Epidemic*. World Health Organization; 1994. https://apps.who.int/iris/handle/10665/58749.
2. *Global Tuberculosis Report 2021*. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
3. Janmeja AK, Aggarwal D, Dhillon R. Factors predicting treatment success in multi-drug resistant tuberculosis patients treated under programmatic conditions. *Indian J Tubercul.* 2018 Apr;65(2):135–139.
4. Gupta A, Chopra V. Evolution of newer regimens in TB from RNTCP to NTEP. *Indian J Tubercul.* 2020 Dec;67(4S):S107–S110.
5. Esposito S, Bianchini S, Blasi F. Bedaquiline and delamanid in tuberculosis. *Expert Opin Pharmacother*. 2015;16(15):2319–2330.
6. Rustomjee R, Diacon AH, Allen J. Early bactericidal activity and pharmacokinetics of the diarylquinoline TMC207 in treatment of pulmonary tuberculosis. *Antimicrob Agents Chemother*. 2008;52:2831–2835.
7. Guidelines for programmatic management of drug resistant tuberculosis in India. https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4780&lid=3306f; 2017.
8. Guidelines for programmatic management of drug resistant tuberculosis in India. https://tbcindia.gov.in/index1.php?lang=1&level=1&sublinkid=4150&lid=2794; 2019.
9. Report of first national anti-tuberculosis drug resistance survey India 2014-2016. https://tbcindia.gov.in/index1.php?lang=1&level=1&sublinkid=4150&lid=2794; 2016.
10. K B, Singla R, Singla N, et al. Factors affecting the treatment outcome of injection based shorter MDR-TB regimen at a referral centre in India. *Monaldi Arch Chest Dis*. 2022 Oct 5;93(3).
11. Jain K, Desai M, Solanki R, Dikshit RK. Treatment outcome of standardized regimen in patients with multidrug resistant tuberculosis. *J Pharmacol Pharmacother*. 2014 Apr;5(2):145–149.
12. Udwadia ZF, Moharil G. Multidrug-resistant-tuberculosis treatment in the Indian private sector: results from a tertiary referral private hospital in Mumbai. *Lung India*. 2014 Oct;31(4):336–341.
13. Yadav AK, Mehrotra AK, Agnihotri SP, Swami S. Study of factors influencing response and outcome of Cat-IV regimen in MDRTB patients. *Indian J Tubercul*. 2016;63(4):255–261.
14. Patel SV, Nimavat KB, Alpesh PB, et al. Treatment outcome among cases of multidrug-resistant tuberculosis (MDR TB) in Western India: a prospective study. *J Infect Public Health*. 2016 Jul-Aug;9(4):478–484.
15. Neeta PN, Prashanth N, Ramaprasad G, Gangadhar Goud T, Sameena AR. A study on outcome of standardized treatment in multi-drug resistance tuberculosis patients. *Int J Community Med Public Health*. 2016;3:257–263.
16. Dole SS, Waghmare VN, Shaikh AM. Clinical profile and treatment outcome of drug resistant tuberculosis patients of Western Maharashtra, India. *JAPI*. 2017;65:18–21.
17. Suryawanshi SL, Shewade HD, Nagaraja SB, Nair SA, Parmar M. Unfavourable outcomes among patients with MDR-TB on the standard 24-month regimen in Maharashtra, India. *Public Health Action*. 2017;7:116–122.
18. Janmeja AK, Deepak S, Dakshayani KR. Analysis of treatment outcome in multi-drug resistant tuberculosis patients treated under programmatic conditions. *Int J Res Med Sci*. 2017;5:2401–2405.
19. Waghmare MA, Utpat K, Joshi JM. Treatment outcomes of drug-resistant pulmonary tuberculosis under programmatic management of multidrug-resistant tuberculosis, at tertiary care center in Mumbai. *Med J DY Patil Univ*. 2017;10:41–45.
20. Parmar MM, Sachdeva KS, Dewan PK, et al. Unacceptable treatment outcomes and associated factors among India's initial cohorts of multidrug resistant tuberculosis (MDR-TB) patients under the revised national TB control programme (2007 – 2011): evidence leading to policy enhancement. *PLoS One*. 2018;13, e0193903.
21. Gupta N, Jorwal P. Treatment outcomes associated with multidrug-resistant tuberculosis. *J Global Infect Dis*. 2018;10:125–128.
22. Singh A, Prasad R, Kushwaha RAS, et al. Treatment outcome of multidrug-resistant tuberculosis with modified DOTS-plus strategy: a 2 years' experience. *Lung India*. 2019 Sep-Oct;36(5):384–392.
23. Takhar RP, Saxena A, Khangarot S, Garg R, Bansiwal B, et al. Treatment outcome of multi-drug resistant tuberculosis(MDR-TB) patients at a tertiary care centre of Northern India. *Eur Respir J Suppl*. 2019;54(63):463.
24. Sharma N, Khanna A, Chandra S, et al. Trends & treatment outcomes of multidrug-resistant tuberculosis in Delhi, India. *A retrospective record-based study. Indian J Med Res*. 2020 Jun;151(6):598–603, 2009-2014.
25. Das M, Dalal A, Laxmeshwar C, Ravi S, Mamnoon F, et al. One step forward: successful end-of-treatment outcomes of patients with drug-resistant tuberculosis who received concomitant bedaquiline and delamanid in Mumbai, India. *Clin Infect Dis*. 2020 Nov;73(9). e3496–e3504.
26. Guglielmetti L, Chiesi S, Eimer J, et al. Bedaquiline and delamanid for drug-resistant tuberculosis: a clinician's perspective. *Future Microbiol*. 2020 Jun;15:779–799.
27. Wang MG, Wu SQ, He JQ. Efficacy of bedaquiline in the treatment of drug-resistant tuberculosis: a systematic review and meta-analysis. *BMC Infect Dis*. 2021 Sep 17;21(1):970.
28. Haley Connie A, et al. Implementation of bedaquiline, pretomanid, and linezolid in the United States: experience using a novel all-oral treatment regimen for treatment of rifampin-resistant or rifampin-intolerant tuberculosis disease. *Clin Infect Dis*. 2023:312.
29. Tiberi S, Utjesanovic N, Galvin J, et al. Drug resistant TB - latest developments in epidemiology, diagnostics and management. *Int J Infect Dis*. 2022 Nov;124:S20–S25.

Indian Journal of Tuberculosis 71 (2024) S72–S76

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Risk factors of treatment interruptions among drug-sensitive and drug-resistant pulmonary tuberculosis patients - A study from South Delhi, New Delhi, India

Lokender Kumar [a], Santosh Kumar [a], Vikram Vohra [a], Krishna Mohan [b], Khalid Umer Khayyam [c,*]

[a] Department of Tuberculosis and Respiratory Diseases, National Institute of TB and Respiratory Diseases, Sri Aurobindo Marg, New Delhi, 110030, India
[b] Division of Clinical Research, Department of Biosciences, School of Basic and Applied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, 203201, India
[c] Department of Epidemiology and Public Health, National Institute of TB and Respiratory Diseases, Sri Aurobindo Marg, New Delhi, 110030, India

ARTICLE INFO

Keywords:
Treatment interruptions
Anti-tuberculosis treatment
DOTS
Default
Non-compliance
Pulmonary tuberculosis
Treatment adherence

ABSTRACT

Background: A variety of factors influence adherence to the lengthy duration of anti-tuberculosis treatment, making it a complicated and dynamic problem. The objective of this study was to investigate the treatment interruption patterns using pre-defined criteria among a cohort of pulmonary tuberculosis patients and to elicit the associated factors.

Methods: This prospective, observational study was conducted between October 2016 to May 2018. All smear and culture positive pulmonary tuberculosis patients (age $\geq$ 14 years) enrolled between October 1, 2016 to March 31, 2017 across 3 Designated Microscopy Centers (DMCs) were included and followed up till end of treatment for outcome in drug-sensitive, and till interim outcome at 6 months for drug-resistant TB patients. Patterns and reasons for interruptions were recorded as per the study protocol.

Results: 171 patients were enrolled in this study, of which 135 (78.94 %) were on Category-I and Category-II treatment (drug-sensitive tuberculosis), 23 (13 %) were multidrug-resistant (MDR) and 13 (8 %) were extensively drug resistant (XDR) tuberculosis patients. Among the drug-sensitive group, 65 (48 %) patients completed their treatment without any interruption while 70 (52 %) patients interrupted with at least one missed dose. Among the 36 MDR/XDR patients, 19 (53 %) patients did not interrupt treatment, but 17 (47 %) patients interrupted with at least one missing dose. The 87 patients in both sub-groups interrupted for 232 times/episodes of which 140 were short and 84 were long interruptions. The main reasons for interruption were found to be busy schedule in 63 (29 %) patients, adverse drug reactions in 40 (18.4 %) and comorbidities in 43 (19.8 %) patients. Feeling of early improvement/no improvement in 23 (10.5 %) patients, addictions in 27 (12.4 %) patients, lack of family support in 14 (6.4 %), unawareness of dosage and duration of treatment in 7 (3.20 %) patients were other common reasons.

Conclusion: The plurality of patients studied were found to be in the younger age group i.e., 14–25 years (n = 75), constituting nearly 44 % of all the patients included and male treatment interrupters (62 %) outnumbered the females (38 %), possibly owing to work schedule or addictions. The majority of interruptions were related to patient related factors (93.5 %), followed by DOTS provided factors (6.40 %) and system related factors (3.01 %). Further studies should be conducted to classify the factors of treatment interruptions in detail and also to study the impact of these interruptions' patterns on final outcomes.

1. Introduction

Mycobacterium tuberculosis is the infectious agent that causes tuberculosis (TB). It is most frequently transmitted when a patient with untreated TB coughs or sneezes, and infected droplet nuclei released into the air are inhaled by other people. Although not everyone who

* Corresponding author. Epidemiology and Public Health, and District TB Officer, NITRD-RNTCP Room No. 19, National Institute of TB and Respiratory Diseases, Sri Aurobindo Marg, New Delhi, 110030, India.
E-mail addresses: l.kumar@nitrd.nic.in (L. Kumar), santosh.nadikatla@gmail.com (S. Kumar), v.vohra@nitrd.nic.in (V. Vohra), krishna.mohan.85@gmail.com (K. Mohan), khalid.k@nitrd.nic.in (K.U. Khayyam).

https://doi.org/10.1016/j.ijtb.2024.01.001
Received 3 November 2022; Received in revised form 9 July 2023; Accepted 3 January 2024
Available online 3 January 2024

contracts the infection goes on to develop TB illness. In immunocompetent adults, the lifetime risk of getting active TB is estimated to be 5%–10 %, while in people with HIV, this risk rises to 5%–15 % annually.[1] India, which has 1.4 million annual TB deaths and contributes for a fourth of the 10 million global TB cases.[2]

The Revised National Tuberculosis Control Program (RNTCP), a national initiative with a staged expansion strategy, was introduced in 1997. The Directly Observed Therapy, Short-course (DOTS) technique was subsequently selected by the RNTCP as the most methodical and economical method for reviving the TB control effort in India.[3] The programme consistently met global benchmarks for case detection and treatment success rates. Drug-resistant TB programmatic management started in 2007, and countrywide coverage was attained in March 2013.

Controlling TB faces numerous difficulties in India. In addition to being crucial for providing quality patient care, early, accurate diagnosis and efficient treatment of TB are also the cornerstones of any programme aimed at reducing tuberculosis cases. Poor adherence to a prescribed treatment for TB, a communicable disease needing lengthy treatment, raises the risk of disease transmission, mortality, and morbidity. When endeavoring to treat a patient or manage diseases in a community, one must take the reality of treatment interruption into account.

Patients who default treatment are at increased risk for development of drug resistance and relapse.[4,5] As non-compliance with treatment may result in TB persistence and resurgence, longer infectiousness, and increased transmission rates, treatment breaks are a major cause for concern.[6,7] In comparison to compliant patients, non-adherent patients need longer duration for treatment and are also less likely to complete the treatment.[8,9]

Despite the implementation of the globally recommended Directly Observed Therapy, Short-course (DOTS) strategy by the RNTCP (now known as National Tuberculosis Elimination Program or NTEP), the biggest obstacle to tuberculosis control is still treatment interruption. It has been estimated that even up to 70 to 90% of patients fail to take their drugs regularly.[9] Treatment interruption is major obstacle to the management of TB and is the most important challenge for TB control.

The National Institute of TB & Respiratory Diseases (NITRD), New Delhi is an autonomous institution highly known for its expertise in diagnosis, treatment, training, and research on respiratory disorders and tuberculosis. It carries out tuberculosis control activities in a specific geographic region in Delhi's southern part and covering a population of roughly 0.9 million. This prospective observational study was carried out in National Institute of TB & Respiratory Diseases, including patients from 03 of the 09 Designated Microscopy Centers or DMCs under its aegis, in order to study patterns of treatment interruptions and the various reasons for their interruptions during the treatment period. To date, there are also limited studies available investigating the risk factors for early treatment interruptions.[10]

2. Methods

The study population chosen were all smear as well as culture positive pulmonary TB patients (age $\geq$ 14 years), registered at three DMC's i.e., Ladosarai, Mehrauli and Khanpur. Ethical clearance was taken from the ethics committee of NITRD, New Delhi (No: NITRD/PGEC/2015/11206). These patients were followed up till final outcome of Category-I and Category-II anti-tubercular treatment (ATT) and till 6 months interim outcome in case of MDR-TB and XDR-TB patients for collecting data on assessment of the interruptions, if any. Details of the patients on demography, age, gender, address, DMC area, type of disease, category, current sputum status, TB number/Nikshay ID, date of starting treatment etc. were noted from TB register, DOTS PLUS register or Nikshay portal. All these parameters were enumerated in a pre-designed proforma. The inclusion and exclusion criteria were as defined below.

2.1. Inclusion criteria

All new and re-treatment smear positive pulmonary tuberculosis patients.

All MDR and XDR PTB Patients diagnosed by Cartridge-based Cartridge Based Nucleic Acid Amplification Test (CBNAAT), Line Probe Assay (LPA) and Mycobacteria Growth Indicator Tube (MGIT).

Patients of both sexes of age 14 years or more.

2.2. Exclusion criteria

Smear negative/culture negative patients.

Children of age less than 14 years.

EPTB patients.

The following definitions were taken from previous studies and used to classify various interruptions.[11,12]

Short term interruption: In drug sensitive patients, if drugs stopped for at least one day in intensive phase and at least one week in continuation phase and in MDR/XDR patients if anti-tuberculous drugs stopped for up to two days.

Long term interruption: In drug sensitive patients, if drugs stopped for more than one day in intensive phase and more than one week in continuation phase and MDR/XDR patients, if ATT is stopped for more than two days.

This was a prospective observational study conducted from October 2016 to May 2018. All smear and culture positive TB patients enrolled in two quarters (i.e., fourth quarter of 2016 and first quarter of 2017) was taken from the three DMCs (Ladosarai, Mehrauli and Khanpur) depending on feasibility of enrolment of cases. Total number of patients registered during the same period of time for the three DMCs in previous two years was observed to be 175 in 4th quarter of 2014 and 1st quarter of 2015; and 160 during 4th quarter of 2015 and 1st quarter of 2016. The required sample size was calculated using the formula $N = \{Z^2_{1-\alpha/2}\}$ (1-P)/ϵ^2 P, where Z is equal to 1.96 (80 % power), expected population proportion (P) was 38 %, relative precision (ϵ) of 20 %, at 95 % confidence interval resulting in a sample of around 160 cases. Consecutive enrolment as per inclusion criteria was done and after data collection and checking for completeness and consistency, the patient's characteristics at treatment initiation, distribution of potential risk factors, categorical variables were summarized using frequencies and percentages and the data were analyzed using SPSS software Version 25.

3. Results

3.1. Baseline characteristics of the enrolled patients

Among the 135 patients of new and re-treatment (drug-sensitive) group 59 (43 %) belonged to age group 14–25 years. Among the 36 patients of MDR/XDR 16 (44 %) belonged to age group 14–25 years. In both groups maximum number of patients belongs to younger age group i.e., 14–25 years (n = 75). 88 (65 %) were male and 47 (35 %) were female. Among 36 patients of MDR/XDR patients 19 (53 %) were male and 17 (47 %) were female. Even though males (n = 107) were predominant in both groups the difference is more in new & re-treatment patients whereas it very less in MDR/XDR patients. According to the modified Kuppuswamy scale (1981), the socioeconomic status was divided into upper, upper middle, lower middle, upper lower, and lower class. It was noted that the majority of study population belong to lower middle class i.e., 81 patients (47 %) in both groups, followed by upper middle with 37 patients (22 %), upper lower with 28 patients (16 %), lower with 16 patients (10 %) and upper with 9 patients (5 %) respectively.

The treatment category assigned along with the other baseline characteristics is shown in Table 1.

Table 1
Baseline characteristics for the included patients.

Parameter	Category I and II [drug sensitive] patients (n = 135)	MDR/XDR patients (n = 36)	Total (n = 171)
Age group (in years)			
14–25	59(43 %)	16(44 %)	75(44 %)
26–35	32(24 %)	9(25 %)	41(24 %)
36–45	16(12 %)	5(14 %)	21(12 %)
>45	28(21 %)	6(17 %)	34(20 %)
Gender			
Male	88(65 %)	19(53 %)	107(63 %)
Female	47(35 %)	17(47 %)	64(37 %)
Education			
Illiterate	39(29 %)	10(28 %)	49(29 %)
Primary school/ Literate	30(22 %)	10(28 %)	40(23 %)
High school	42(31 %)	7(19 %)	49(29 %)
Intermediate	12(9 %)	5(14 %)	17(10 %)
Graduate	7(5 %)	2(6 %)	9(5 %)
Professional/Post-Graduate	5(4 %)	2(6 %)	7(4 %)
Occupation			
Unemployed	36(27 %)	12(33 %)	48(28 %)
Unskilled worker	34(26 %)	7(19 %)	41(24 %)
Semiskilled worker	23(17 %)	9(25 %)	32(19 %)
Skilled worker	23(17 %)	3(8 %)	26(15 %)
Clerk, Shop owner	9(7 %)	4(11 %)	13(8 %)
Semi-professional	7(5 %)	1(3 %)	8(5 %)
Professional	4(3 %)	0(0 %)	4(2 %)
Socio-economic group			
Lower	13(10 %)	3(8 %)	16(10 %)
Upper lower	22(16 %)	6(17 %)	28(16 %)
Lower middle	62(46 %)	19(53 %)	81(47 %)
Upper middle	30(22 %)	7(20 %)	37(22 %)
Upper	8(6 %)	1(2 %)	9(5 %)
Addictions (Smoking or Alcohol)			
Yes	30(22 %)	8(22 %)	38(22 %)
No	105(78 %)	28(78 %)	133(78 %)
Treatment Category			
CAT-I	99 (58 %)	NA	99 (58 %)
CAT-II	36 (21 %)	NA	36 (21 %)
CAT-IV	NA	23 (13 %)	23 (13 %)
Pre-XDR/XDR Regimen	NA	13 (8 %)	13 (8 %)

3.2. Treatment interruptions

Table 2 shows that, among 135 CAT-I and CAT-II patients, 65 (48 %) completed their treatment without any interruption during treatment and in 70 (52 %) patients' interruption in at least one dose during their treatment was seen.

Among 70 patients who interrupted at least one time, 23 (33 %) patients were interrupted during INTENSIVE PHASE, 37 (53 %) patients were interrupted during CONTINUATION PHASE and 10 (14 %) were interrupted during both the phases, as shown in Table 3.

In the MDR and XDR sub-group, there were 17 (47 %) patients in whom the treatment was interrupted at least once, whereas in 19 (53 %) there was no interruption in treatment till the interim treatment outcome of 06 months, Table 4.

Table 2
CAT-I & CAT-II treatment interruptions.

	Male	Female	Total (%)
Patients without any treatment interruption	42(65 %)	23(35 %)	65(48 %)
Patients with at least one or more treatment interruption	46(66 %)	24(34 %)	70(52 %)
Total (%)	88(66 %)	47(34 %)	135(100 %)

Table 3
CAT-I & CAT-II Phase-wise interruptions.

Phase wise interruptions	Frequency (n = 70)	%
In Intensive phase (IP)	23	33 %
In Continuation phase (CP)	37	53 %
In both IP and CP	10	14 %
Total	70	100 %

3.3. Reasons affecting interruption

Various reasons for each interruption were noted and tabulated into four categories as shown in Table 5. Among the total 171 patients studied, 87 patients (70 CAT-I & II, 17 MDR/XDR) had interruptions during their treatment. These 87 patients were interrupted 232 times/ episodes. The breakdown of the factors in the assessments were tabulated into four categories: patient factors; DOT provider factors; system factors; and environmental factors. The majority of reasons for their interruption were related to patient related factors (93.5 %). Table 5 also shows frequency of the factors associated with each category of interruption.

The most common reason for interruption, 63 patients (29 %) were due to their schedule like visit required to their village, office work, busy at occupation etc. Uncontrolled comorbidities, 43 patients (19.80 %) and adverse effects of drugs/intolerance to drugs, 40 patients (18.40 %) and were found to be second and third most common reasons for interruptions in our study, respectively. Early improvement/no improvement (10.5 %), addictions (12.40 %), lack of family support (6.40 %) and unawareness of dosage and severity of disease (3.20 %) were other common reasons elicited by patients in our study for the interruptions.

Among 15 (6.4 %) DOTS provider factors, the majority of 8 (3.45 %) incidents were because of non-recording in DOTS Treatment Card by treatment provider, which were not actually interruptions in the dose or treatment. Not giving the proper instructions to the patients about dosage and schedule is also one of the reasons for treatment interruptions.

System factors were contributing very less role in treatment interruptions. Among 7 (3.01 %) incidences of interruption, 5 (2.15 %) were due to RNTCP strike in Delhi and 2 (0.86 %) were due to non-availability of drugs at the respective center. In our study, we did not find any environmental factors like rain, floods etc. as a reason for interruptions, this is because the study population was within the reach of DOTS centers and with good transport facilities.

4. Discussion

A study by Singh et al., 2013 reported findings observed in their study, in which out of 80 treatment interrupters, majority of patients, i. e., 50 (62.5 %) interrupted treatment in the continuation phase.[9] In our study the percentage defaulters who interrupted treatment in the continuation phase was seen to be 53 %. Reason for higher rate of interruptions in continuation phase could be feeling of early improvement by the patient and needs to be investigated further.

In the MDR and XDR sub-group, almost half of the study population

Table 4
MDR & XDR treatment interruptions.

	Male	Female	Total (%)
Patients without any treatment interruption	11(57 %)	8(43 %)	19 (53 %)
Patients with at least one or more treatment interruption	8(47 %)	9(53 %)	17 (47 %)
Total (%)	19(52 %)	17(48 %)	36 (100 %)

Table 5
Distribution of the factors associated with treatment interruptions.

Factors	Frequency/Episodes (n = 232)	Percent (%)
Patient factors	*217*	*93.50 %*
Adverse event/intolerance of drugs	40	18.40 %
Uncontrolled comorbidities	43	19.80 %
Busy schedules	63	29.00 %
Lack of family support	14	6.40 %
Financial crisis	0	0 %
Unawareness of dosage and duration of schedule	7	3.20 %
Addictions	27	12.40 %
Early improvement/no improvement	23	10.5
DOTS Provider factors	*15*	*6.40 %*
DOTS Provider on leave	0	0 %
Behavior of DOTS provider	0	0 %
Time taking	0	0 %
Proper instructions not given	7	3.01 %
No recording	8	3.45 %
System factors	*7*	*3.01 %*
Non-availability of drugs	2	0.86 %
Availability of expired drugs	0	0 %
Center timings	0	0 %
RNTCP strike	5	2.15 %
Environmental factors	*0*	*0 %*

missed the at least one dose of ATT during the treatment period. However, in another study by Jamneja et al., 2017, interruption rates in MDR tuberculosis patients were reported at around 9.3 %.[13] This difference can be attributed to the fact that missing of a single dose of ATT in our study was taken as an interruption while missing of more than 10% of the prescribed doses, was considered as non-adherent or default in the other study. Also, the latter did not include XDR-TB patients as the current study did. XDR-TB patients have much higher default rate than MDR-TB patients and could also explain our high interruption rates.

A study by Kaona et al., 2004 had found that 38.6 % of the non-compliant or default TB patients stopped taking their anti-tubercular medication once they started feeling better.[14] A similar study also identified social issues and a sense of improvement as the top two causes of patient default.[15] A study by Jaggarajamma et al., 2007 also found that in addition to drug-related problems and migration, the early relief from symptoms were one of the common reasons for default as reported by the patients (20 %).[16] This is contrast to our study (10 %), and it can be because former study mostly reporting the reasons for default, whereas in this study the reasons for interruptions during the treatment period were studied. Further the DMC's included in our study area come under National Institute of Tuberculosis and Diseases, so the patients were already well counseled for the treatment course and severity of disease etc. Whereas 61 % of non-adherent patients in a Kathmandu based study by Bam et al., 2006, claimed they were unaware of the necessity to continue or complete their therapy, particularly after feeling better.[17]

Side effects from ATT were ranked as the most frequent causes of therapy cessation and have also been reported as one of the leading causes.[18–20] In another study by Jakubowiak et al., 2006, alcohol use was regarded as one of the most frequent causes of treatment discontinuation, while in this study addictions were the fifth most common reason.[21]

Similarly, patients default to therapy due to inadequate counselling, poor service delivery, and providers' negative attitudes. The majority of patients forego treatment, according to a case control study done in Rio de Janeiro, Brazil, by Salles et al., 2004, because they don't feel comfortable with doctors, their blood pressure not tested, and health-care personnel don't give them cards with the review date on them.[22]

5. Conclusion

Poor compliance to treatment is considered one of the most serious problems of TB control in India as patients who default treatment are at increased risk for development of drug resistance and relapse. Shorter interruptions of treatment are also a point of concern as non-compliance with treatment may lead to persistence and resurgence of TB, prolonged infectiousness, and increased transmission rates. Non-adherent patients require longer periods of treatment and are less likely to complete treatment compared to patients who are compliant.

In this study, the patients of three DMC's, which represent the urban population were studied, and these are under continuous supervision of National Institute of TB & Respiratory Diseases, thus making it difficult to generalize the results to all patients of other regions and rural centers. The controls were not taken, and the sample size is small due to limited study period, further field studies are required on a large scale to generalize the results of this study. The MDR/XDR patients were followed up till interim outcome i.e., 6 months, so we are unable to find the impact of these treatment interruption patterns on final outcome.

Financial support and sponsorship

None.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgement

The authors are highly grateful to the Department of Tuberculosis and Respiratory Diseases, National Institute of Tuberculosis and Respiratory Diseases, New Delhi for providing necessary logistics, approvals and support for the conduct of this study.

References

1. Aaron L, Saadoun D, Calatroni I, et al. Tuberculosis in HIV-infected patients: a comprehensive review. *Clin Microbiol Infect*. 2004 May;10(5):388–398. https://doi.org/10.1111/j.1469-0691.2004.00758.x.
2. World Health Organization. *–232. Global Tuberculosis Report 2020*. Geneva: WHO; 2020:1. Available online at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports. Accessed April 21, 2022.
3. Purty AJ. Detect–treat–prevent–build: strategy for TB elimination in India by 2025. *Indian J Commun Med*. 2018 Jan–Mar;43(1):1–4. https://doi.org/10.4103/ijcm.IJCM_321_17.
4. Dooley KE, Lahlou O, Ghali I, et al. Risk factors for tuberculosis treatment failure, default, or relapse and outcomes of retreatment in Morocco. *BMC Publ Health*. 2011 Feb 28;11:140. https://doi.org/10.1186/1471-2458-11-140.
5. Kabir S, Junaid K, Rehman A. Variations in rifampicin and isoniazid resistance associated genetic mutations among drug naïve and recurrence cases of pulmonary tuberculosis. *Int J Infect Dis*. 2021 Feb;103:56–61. https://doi.org/10.1016/j.ijid.2020.11.007.
6. Nahid P, Jarlsberg LG, Rudoy I, et al. Factors associated with mortality in patients with drug-susceptible pulmonary tuberculosis. *BMC Infect Dis*. 2011 Jan 3;11:1. https://doi.org/10.1186/1471-2334-11-1.
7. Vree M, Huong NT, Duong BD, et al. Mortality and failure among tuberculosis patients who did not complete treatment in Vietnam: a cohort study. *BMC Publ Health*. 2007 Jul 2;7:134. https://doi.org/10.1186/1471-2458-7-134.
8. Davidson H, Schluger NW, Feldman PH, Valentine DP, Telzak EE, Laufer FN. The effects of increasing incentives on adherence to tuberculosis directly observed therapy. *Int J Tubercul Lung Dis*. 2000 Sep;4(9):860–865.
9. Singh A, Bhardwaj A, Mukherjee AK, Arya R, Mithra P. Current status of timing of treatment interruption and pattern of default among tuberculosis patients on directly observed treatment. *J NTR Univ Health Sci*. 2013;2:177–180.
10. Suliman Q, Lim PY, Said S Tan Md, Kt, Mohd, Zulfeki NA. Risk factors for early TB treatment interruption among newly diagnosed patients in Malaysia. *Sci Rep*. 2022; 12:745. https://doi.org/10.1038/s41598-021-04742-2.
11. Bastard M, Sanchez-Padilla E, Hewison C, et al. Effects of treatment interruption patterns on treatment success among patients with multidrug-resistant tuberculosis in Armenia and Abkhazia. *J Infect Dis*. 2015 May 15;211(10):1607–1615. https://doi.org/10.1093/infdis/jiu551.
12. Driver CR, Matus SP, Bayuga S, Winters AI, Munsiff SS. Factors associated with tuberculosis treatment interruption in New York City. *J Public Health ManagPract*. 2005 Jul-Aug;11(4):361–368. https://doi.org/10.1097/00124784-200507000-00017.
13. Janmeja AK, Aggarwal D, Dhillon R. Analysis of treatment outcome in multi-drug resistant tuberculosis patients treated under programmatic conditions. *Int J Res Med*

Sci. 2017 May 27;5(6):2401–2405 [Internet] https://www.msjonline.org/index.php/ijrms/article/view/3317.
14. Kaona FA, Tuba M, Siziya S, Sikaona L. An assessment of factors contributing to treatment adherence and knowledge of TB transmission among patients on TB treatment. *BMC Publ Health*. 2004 Dec 29;4:68. https://doi.org/10.1186/1471-2458-4-68.
15. Demissie M, Kebede D. Defaulting from tuberculosis treatment at the addis abeba tuberculosis centre and factors associated with it. *Ethiop Med J*. 1994 Apr;32(2): 97–106.
16. Jaggarajamma K, Sudha G, Chandrasekaran V, et al. Reasons for non-compliance among patients treated under revised national tuberculosis control programme (RNTCP), tiruvallur district, South India. *Indian J Tubercul*. 2007;54(3):130–135.
17. Bam TS, Gunneberg C, Chamroonsawasdi K, et al. Factors affecting patient adherence to DOTS in urban Kathmandu, Nepal. *Int J Tubercul Lung Dis*. 2006;10(3): 270–276. 18.
18. Gupta Sweta, Gupta Sanjay, Behera Digamber. Reasons for interruption of anti-tubercular treatment as reported by patients with tuberculosis admitted in a tertiary care institute Indian. *J Tuberc*. 2011;58:11–17.
19. Wares DF, Singh S, Acharya AK, Dangi R. Non-adherence to tuberculosis treatment in the eastern Tarai of Nepal. *Int J Tubercul Lung Dis*. 2003 Apr;7(4):327–335.
20. Tekle B, Mariam DH, Ali A. Defaulting from DOTS and its determinants in three districts of Arsi Zone in Ethiopia. *Int J Tubercul Lung Dis*. 2002 Jul;6(7):573–579.
21. Jakubowiak WM, Bogorodskaya EM, Borisov SE, Danilova ID, Lomakina OB, Kourbatova EV. Social support and incentives programme for patients with tuberculosis: experience from the Russian Federation. *Int J Tubercul Lung Dis*. 2007; 11(11):1210–1215.
22. Salles CL, Conde MB, Hofer C, et al. Defaulting from anti-tuberculosis treatment in a teaching hospital in Rio de Janeiro, Brazil. *Int J Tubercul Lung Dis*. 2004 Mar;8(3): 318–322.

Indian Journal of Tuberculosis 71 (2024) S77–S80

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Mutational analysis in drug resistant *Mycobacterium tuberculosis* in Western Uttar Pradesh

Mamta Mishra, PhD [a], Anita Pandey [a,*], Priyanka Chaturvedi [a], Eema Chaudhary [b], Arjun Singh Bisht [a]

[a] Department of Microbiology, Subharti Medical College, Swami Vivekanand Subharti University Meerut, UP, India
[b] Department of TB & Chest, Subharti Medical College, Swami Vivekanand Subharti University Meerut, UP, India

ARTICLE INFO

Keywords:
MDRTB
Mutational analysis
Line probe assay
Pulmonary TB

ABSTRACT

Background: Multi-drug resistant tuberculosis (MDR-TB) results in treatment failure and poor clinical outcomes. This study was carried out with the aim to determine the pattern of drug resistance against *Mycobacterium tuberculosis* towards first line ATT (anti-tubercular treatment) in sputum smear–positive patients using Line Probe Assay (LPA).

Methods: A cross sectional prospective study was carried out in a tertiary care Hospital of Meerut. A total of 898 sputum samples (on spot and early morning) collected from 449 suspected pulmonary tuberculosis patients as per RNTCP guidelines were screened by microscopy. Decontamination was done by N-acetyl-L-cysteine and sodium hydroxide. Then smear positive samples were subjected to 1st line drug susceptibility testing (DST) using LPA GenoType® MTBDRplus (HAIN Life Science) assay, a molecular method which allows rapid detection of Rifampicin (Rif) and Isoniazid (INH) resistance.

Results: The overall burden of MDR TB in this geographical area was 7.9 %. Mono-resistance with Rif alone was around 2.8 %. However, the mono-resistance with INH (inhA gene) and INH (katG gene) was 2.8 % and 1.1 % respectively. Drug resistance of Rif was due to mutations in rpoB gene while resistances to INH were more commonly due to mutation in inhA gene followed by katG gene. TB was more commonly seen in the age group of 30–59 years (43.8 %) and predominantly in males.

Conclusion: Tuberculosis positivity rate is high in Western Uttar Pradesh. Burden of MDR TB in Western Uttar Pradesh was similar to National data. Line probe assay can be used as a primary method to diagnose multi drug resistant TB as done in present study which can help in earlier initiation of correct therapy.

1. Introduction

Mycobacterium tuberculosis primarily affects lungs and causes pulmonary tuberculosis (PTB) but also other parts of the body such as reproductive organs, spine etc termed as extra-pulmonary TB (EPTB). Partial or untreated disease may start with slow destruction of the lungs, ultimately leading to death.[1] Isoniazid (INH) is the bactericidal agent that ensures early clinical recovery and helps in reduction of transmission of tuberculosis. However, prevention of relapse is done by Rifampicin (Rif), a potent myco-bactericidal drug.[1] Multi-drug resistant TB (MDR-TB) patients are defined as those which confer resistance to two important first line anti-tubercular treatments (ATT) namely Isoniazid (INH) and Rifampicin (Rif). MDR-TB results in treatment failure and poor clinical outcomes and has only 60 % chance to get complete cure.[2] Term extensively drug resistant TB (XDR-TB) was used for the first time in March 2006.[3] The XDR-TB ultimately is the reason for high morbidity and mortality due to tuberculosis.

As per estimation of World Health Organization (WHO) report 2018, India has the highest burden of TB and MDR-TB in the world. Among the MDR-TB cases, 2.84 % were newly diagnosed patients and 11.60 % were previously treated patients as per first national drug resistance survey.[4] Worldwide, approximately 9.7% of MDR-TB cases get converted into XDR-TB cases, as reported by WHO in 2015.[5] Line probe assay (LPA) is a DNA strip-based test, version 1.0 has been endorsed by WHO in 2008 while version 2.0 in 2011, to determine the mutational analysis of drug resistant *M. tuberculosis*.[6]

* Corresponding author.
E-mail addresses: mansianya@gmail.com (M. Mishra), anipanmicro@gmail.com (A. Pandey), chaturvedi.priyanka2410@gmail.com (P. Chaturvedi), eema.dr@gmail.com (E. Chaudhary), arjunbisht1995@gmail.com (A.S. Bisht).

https://doi.org/10.1016/j.ijtb.2024.01.009
Received 30 December 2022; Received in revised form 1 September 2023; Accepted 14 January 2024
Available online 19 January 2024

This study was carried out with the aim to determine the pattern of drug resistance of *M. tuberculosis* towards first line ATT (anti-tubercular treatment) in patients with smear–positive sputum sample using Line Probe Assay (LPA) at tertiary care hospital of Western Uttar Pradesh.

2. Materials and methods

This cross-sectional prospective study was carried out at tertiary care hospital of Western Uttar Pradesh over a period of one year. Total 3145 patients attended the chest OPD, out of which total 898 sputum samples (on spot and early morning) were collected from 449 suspected cases of Pulmonary tuberculosis, who attended chest OPD and having symptoms of cough with expectoration for more than 2 weeks with other cardinal symptoms of tuberculosis such as weight loss, hemoptysis, anorexia, evening rise of temperature etc. The samples were liquefied by N-acetyl-L-cysteine and decontaminated by 2%NaOH.[6] Smear were prepared with the help of Ziehl Neelsen (ZN) stain followed by direct microscopy was used for grading as per RNTCP guidelines.[7]

All the patients with age ≥18 years irrespective of gender with sputum smear positive for acid fast bacilli, who had given the consent, were included in the study. While patient age <18 years, sputum smear-negative for acid fast bacilli, those who did not give consent, terminally ill patients, inadequate sputum sample, pregnant females and patients with exclusively EPTB were excluded from the study.

Total 178 smear positive sputum samples (RNTCP grade 1+ to 3+, as per the LPA manufacturer's instructions) were subjected to DNA extraction, amplification and 1st line drug susceptibility testing (DST) using LPA GenoType® MTBDRplus (HAIN Life Science) assay, a molecular method which allows rapid detection of Rifampicin (Rif) and Isoniazid (INH) resistance.[8] However, 13 samples graded as scanty positive were not included for DST. The result and interpretation of molecular assay was done as per manufacturer's instructions of LPA using GenoTypeMTBDRplus (Hain Life Science GmbH, Nehren, Germany).[8]

Informed consent was obtained from each patient before enrolling them into study followed by collection of clinical samples.

3. Results

3.1. Sputum positivity rate

The sputum positivity rate in this geographical area was 39.64 % (178/449). Among the sputum positive samples subjected to direct drug susceptibility testing (DST) majority of the samples were grade 1+, followed by 2+ and 3 + [Fig. 1]. Majority of the patients visiting our hospital were newly diagnosed cases (70 %) as compared to previously treated patients (30 %).

3.1.1. Geographical area wise distribution

The positive cases visiting our hospital mainly belong to the surrounding area/villages of the Meerut is 95 (53.37 %) as compared to patients belong to Meerut city is 83 (46.62 %) [Fig. 2].

3.1.2. Age and gender wise distribution

Overall tuberculosis was most common in the age group of 30–59 years (43.8 %) followed by 18–29 years (29.2 %). The positivity rate was high in males (64.04 %) as compared to females (35.95 %). The male: female ratio was 1.78:1. The relationship of age with gender was found to be statically significant (p = 0.000). [Table 1].

3.1.3. Mutational analysis of sputum samples for first line ATT using LPA

The overall prevalence of MDR-TB from this geographical area was 7.9 %. On mutational analysis of various genes encoding first line drugs using LPA; mono-resistance with Rif

i.e. mutation in only rpoB gene was detected in 2.8 % cases. Mono-resistance to INH i.e.mutation to katG gene (drug resistance on high level INH) was found 1.1 % and mutation in inhA gene (drug resistance on low level of INH) was found in2.8 %. [Table 2].

3.2. Pattern of genetic mutations in drug resistant patients

The common cause of drug resistance of Rif that is mutations in rpoB gene was mostly present in WT8 band of LPA strip (530-533region) 47.36 % followed by WT3 band (513–517 region) 31.57 % and WT7 band (526–529) 26.31 %.

However, in case of INH resistance, most common cause of drug resistance was due tomutation in inhA gene as compared to mutation in katG gene. Among inhA gene, the c-15t region of WT1 band showed maximum mutation (47.06 %) followed by t-8c region of WT2 band (35.29 %). In katG gene mutation was only seen in S315T1 region (100 %) of MUT1 band [Table 3].

4. Discussion

The sputum positivity rate in the present study was (178/449) 39.64 %. There was male predominance (64 %) with the male: female ratio of 1.78:1 which is concordant to the study carried out at BHU Varanasi.[9] In our study, the sputum positivity rate was high in age groups of 30–59 years as compared to Sinha et al.[9] where the positivity was high in age group of 21–45 years.

Our prevalence of MDR-TB cases from this Meerut and surrounding area was 7.9 %. Much higher prevalence of 53 %, 34.5 %, 25.8 %, 18.9 %, 18.4 % and 11.42 % have been reported from other parts of the country like Pune,[10] Lucknow,[11] Delhi,[12] Jaipur,[13] Aligarh[14] and Jodhpur[15] respectively. Similarly, higher prevalence of MDR TB (37.3 %) has also been reported by Eldirdery et al.[16] from Sudan. Similarly, low prevalence of MDR TB cases (5 %) have been reported in a recent study done in 2021 in Ethiopia[17] using MTBDRplus version 2.0 LPA in which they found that among MDR-TB cases, 19.8 % were resistant to INH and 9.5 % were Rif resistant. Codon 315 of katG gene and codon 15 of inhA gene had shown dominant genetic mutations. Eldirdery et al.

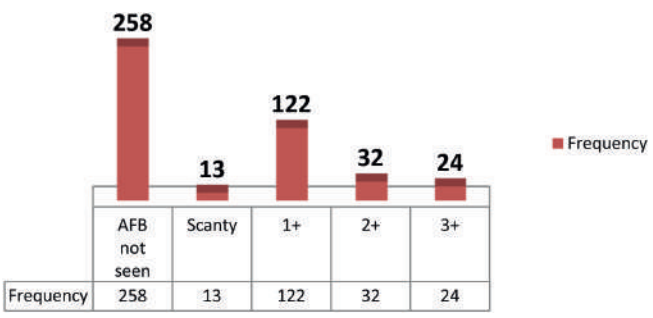


Fig. 1. Distribution of AFB positivity rate and grading of positive samples (n = 449).

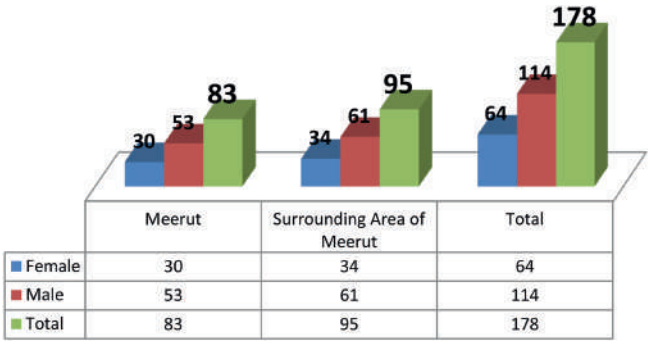


Fig. 2. Geographical area wise distribution of patients with tuberculosis (n = 178).

Table 1
Age and gender wise distribution of tuberculosis patients (n = 178).

		Gender		Total	Chi square = 16.348
		Male	Female		
Age	**18 – 29**	22	30	52 (29.2%)	**DF=2**
	30 – 59	54	24	78 (43.8%)	**P value= 0.000**
	>60	38	10	48 (27.0%)	
Total		**114 (64.04%)**	**64 (35.95%)**	**178 (100.0%)**	

Table 2
Mutational analysis of sputum samples against first line ATT using LPA (n = 178).

LPA	Frequency	Percentage
Sensitive	152	85.4 %
Both INH & Rif Resistant (MDR TB)	14	7.9 %
Mutation in rpoB gene only (Mono-Rifampicin Resistant)	5	2.8 %
Mutation in katG gene only *(drug resistance on high level INH)*	2	1.1 %
Mutation in InhA gene only *(drug resistance at low level INH)*	5	2.8 %
Total	**178**	**100 %**

Table 3
Pattern of genetic mutations in drug resistant patients detected by first line LPA.

Gene	Target Band	MTBDRplus probe	Mutation or Region interrogated	No. (Percentage)
Rifampicin (Rif)				
rpoB gene (n = 19)	WT3	MUT1 developed	D516V	6 (31.6 %)
	WT7	MUT2A developed	H526Y	3 (15.8 %)
	WT7	MUT2B developed	H526D	1 (5.3 %)
	WT8	MUT3 developed	S531L	9 (47.3 %)
Isoniazid (INH)				
katG gene (n = 4)	WT	MUT2 developed	S315T2	4 (100 %)
InhA gene (n = 17)	WT1	MUT1 developed	c-15t	9 (53.0 %)
	WT1	MUT2 developed	a-16 g	3 (17.6 %)
	WT2	MUT3A developed	t-8c	5 (29.4 %)

found that Genotype MTBDRplus had a better sensitivity and specificity to diagnose resistance against INH, Rif and MDR.[16] Overall, mono drug resistance of first line ATT was 14.8 %.

In our study, among the resistant cases, mono-resistance with Rif (mutation in rpoB gene) was detected in 2.8 %, while mono resistance to katG gene and inhA gene showing resistance to INH was 1.1 % and 2.8 % respectively [Table 2]. Similarly, a study conducted at a tertiary care centre in Jodhpur, Rajasthan also reported low prevalence of mono-Rif resistance and mono-INH resistance of 4.28 % and 6.42 % respectively.[15] However, another study conducted by Rufai et al. from AIIMS, New Delhi reported higher prevalence of mono-Rif resistance and mono-INH resistance in 22.2 % and 10.3 % respectively.[12] High positivity from AIIMS may be because it is a tertiary care referral centre where they receive cases from all over the country. In the present study the highest number of Rifampicin drug resistance (47.3 %) i.e. mutations in rpoB gene, was due to lack of WT8 band (530-533region) and presence of mutation in corresponding S531L region followed by missing of WT3 band (513-517region) and development of corresponding D516V region which was present in 31.6 % of overall mutation in rpoB gene [Table 3].

Similarly, the most common cause of drug resistance in INH was due to mutation in inhA gene followed by katG gene. In inhA gene, appearance of c-15t region and corresponding missing of WT1 band showed maximum mutation (53 %) followed by appearance of t-8c region with absence of WT2 band seen in 29.4 %. However, mutations in katG gene were due to appearance of S315T1region with lack of WT band in all the cases (100 %).

Similarly, study done in Sudan in 2016, showed high Rif resistant samples i.e. (83.9 %), were linked to 530–533 codon of rpoB gene while all the samples of INH resistant were linked with codonS315T1 of katG gene.[16] Unlike our study, a study published previously by Jain et al.[11] from KGMU, Lucknow had reported the most common codon of mutant gene rpoB, katG, inhA were S531L, 315 and C15t respectively. Yacoob et al. from Kerala also observed that S531L region of rpoB gene and C15t in the promoter region of inhA gene were most common region showing mutation against Rifampicin and INH resistance respectively.[18]

Gupta et al., in 2014 had concluded that recognition of MDR-TB cases on real time basis, routine surveillance of resistance to anti-TB drug has to be done.[19] However, without are liable diagnostic tool like LPA, it is difficult to find MDRTB cases and hence fight against MDR-TB when end TB strategy is already on war foot mode. The LPA is a good method with sensitivity, specificity, positive predictive value of 96 %, 99 % and 99 % respectively. Moreover, MDRTB plus kit has a definite advantage of being a comparatively rapid method designed to detect genetic mutations in rpoB gene, katG gene and inhA gene in *M. tuberculosis* isolates within 8 h[20] leading to early and correct initiation of ATT.

5. Limitation

The study has few limitation due to limited resources, i) Study needs to be carried out in larger sample size over a longer duration. ii) Those sputum which were positive (scanty) were not included as it had to be grown in culture before subjecting to direct DST. iii) Second line DST using LPA was not performed. However, in future study will be carried out performing 2nd line DST in 1st line resistant cases.

6. Conclusion

Tuberculosis positivity rate is high in Western Uttar Pradesh which can be a hindrance to End TB goal by 2030. Burden of MDR TB in Western Uttar Pradesh was similar to National data. Sputum positivity rate was higher among males as compared to female population. Line probe assay can be used as a primary method to diagnose MDR TB as done in present study which can help in earlier initiation of correct therapy because of its rapid turnaround time.

Source of funding

None.

Ethical approval

Approval from the Institutional Ethics committee was obtained before the commencement of the study via letter no: SMC/IEC/2019/91/02, dated: March 04, 2019.

Declaration of competing interest

None declared.

References

1. Text book of social and preventive medicine. In: Park K, et al., eds. *Jabalpur*. 24th ed. M/S Banarsidas Bhanot Publishers; 2017:191.

2. Engel NC. The making of a public health problem: multi-drug resistant tuberculosis in India. *Health Pol Plann*. 2013;28(4):375–385.
3. Jain A, Mondal R. Extensively drug-resistant tuberculosis: current challenges and threats. *FEMS Immunol Med Microbiol*. 2008;53(2):145–150.
4. India TB report. Ministry Of Health & Family Welfare (MoHFW), GOI, India. Available from: https://tbcindia.gov.in/WriteReadData/l892s/6250311444TB%20India%20Report%202018.pdf; 2018.
5. World Health Organization. *Global Tuberculosis Report 2015*. World Health Organization; 2015:192. Available from: https://apps.who.int/iris/handle/10665/191102.
6. RNTCP DOT Plus, Ministry Of Health & Family Welfare (MoHFW), GOI, India. https://main.mohfw.gov.in/Organisation/Departments%20of%20Health%20and%20Family%20Welfare/revised-national-tuberculosis-programme.
7. Central TB Division. Directorate general of health services. Ministry of Health & Family Welfare, Government of India. Revised national tuberculosis control programme: Guidelines for programmatic management of drug resistant TB (PMDT) in India. Available from: https://tbcindia.gov.in/WriteReadData/l892s/8320929355Guidelines%20for%20PMDT%20in%20India%20-%20May%202012.pdf.
8. Hain lifescience. Your partner in laboratory diagnostics. Available from: https://www.hain-lifescience.de/en/.
9. Sinha P, Srivastava GN, Gupta A, Anupurba S. Association of risk factors and drug resistance pattern in tuberculosis patients in North India. *J Global Infect Dis*. 2017;9(4):139–145.
10. Kagal SDA. Patterns of TB drug-resistance in a tertiary care facility in Pune, India. *Clin Microbiol Open Access*. 2012;2(6). Available from: https://www.academia.edu/55003799/Patterns_of_TB_Drug_Resistance_in_a_Tertiary_Care_Facility_in_Pune_India.
11. Jain A, Singh PK, Chooramani G, Dixit P, Malhotra HS. Drug resistance and associated genetic mutations among patients with suspected MDR-TB in Uttar Pradesh, India. *Int J Tubercul Lung Dis*. 2016;20(7):870–875.
12. Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant Mycobacterium tuberculosis. *J Clin Microbiol*. 2014;52(6):1846–1852.
13. Sharma S, Hanif M, Chopra KK, et al. Detection of multidrug resistance and extensively drug resistance among smear-negative extrapulmonary tuberculosis cases in a reference laboratory. *Biomed Biotechnol Res J (BBRJ)*. 2018;2(2):132. Available from: https://www.researchgate.net/publication/326206317.
14. Ahmed S, Shukla I, Fatima N, Varshney SK, Shameem M, Tayyaba U. Profile of drug-resistant-conferring mutations among new and previously treated pulmonary tuberculosis cases from Aligarh region of Northern India. *Int J Mycobacteriol*. 2018;7(4):315–327.
15. Kumar D, Bohra GK, Meena D. Study of the prevalence of Multidrug-Resistant Pulmonary Tuberculosis (MDR-TB) in Western Rajasthan using line probe assay. *J Fam Med Prim Care*. 2020;9(2):1093–1097.
16. Eldirdery MM, Elrayah IE, Elkarim MOA, et al. Rapid detection of multi drug resistant- tuberculosis using line probe assay (LPA) in Sudan. *Eur Acad Res*. 2016;3(10):10755–10768.
17. Yigzaw WB, Torrelles JB, Wang SH, Tessema B. Magnitude of phenotypic and MTBDRplus line probe assay first-line anti-tuberculosis drug resistance among tuberculosis patients; Northwest Ethiopia. *Infect Drug Resist*. 2021;14:497–505.
18. Yacoob FL, Philomina JB, Lelitha SDK, 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. *J Pathog*. 2016;2016, 6235618.
19. Gupta A, Nagaraja MR, Kumari P, et al. Association of MDR-TB isolates with clinical characteristics of patients from Northern region of India. *Indian J Med Microbiol*. 2014;32(3):270–276.
20. Raizada N, Sachdeva KS, Chauhan DS, et al. A multi-site validation in India of the line probe assay for the rapid diagnosis of multi-drug resistant tuberculosis directly from sputum specimens. *PLoS One*. 2014 Feb 19;9(2), e88626. https://doi.org/10.1371/journal.pone.0088626.

Indian Journal of Tuberculosis 71 (2024) S81–S85

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Pyrazinamide resistance due to *pncA* gene mutation and its association with treatment outcome among tuberculosis patients of South India- A longitudinal observational study

Andrew Marie Xavier V [a], Mirunalini R [b,*], Noyal Mariya Joseph [c], Kalaiarasan Ellappan [c], Muthaiah Muthuraj [d]

[a] *Department of Pharmacology, Kalapet, Pondicherry Institute of Medical Sciences, Puducherry, India*
[b] *Institute block, Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research Institute, Puducherry, India*
[c] *Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education & Research, Gorimedu, Puducherry, India*
[d] *Head of Department, State Intermediate Reference Laboratory, Dhanvanthri nagar, Gorimedu, Puducherry, India*

ARTICLE INFO

Keywords:
Pulmonary tuberculosis
Pyrazinamide resistance
Genotypic resistance
Phenotypic resistance
Pyrazinoic acid

ABSTRACT

Introduction: Mycobacterium tuberculosis has been extensively studied for mutations leading to drug resistance. Pyrazinamide is a drug acting on the semi-dormant bacteria that is responsible for relapse of tuberculosis. This drug helped reduce the treatment duration of tuberculosis from nine to six months. However, this drug is not being screened for resistance along with Rifampicin and Isoniazid.

Aims and objectives: This study aimed to estimate the proportion of *pncA* gene mutation among tuberculosis patients and its association between treatment outcomes, clinical characteristics, and phenotypic drug resistance.

Method: ology: A total of 154 samples included 73 drug-resistant and 81 drug-susceptible isolates. The isolates were subjected to DNA extraction and amplification using conventional PCR. The PCR product was sequenced by the Sanger sequencing method, and phenotypic drug susceptibility testing was done using the broth dilution method. The association of this gene with the treatment outcome was done by following up with the patients till the end of the regimen.

Results: None of the drug susceptible tuberculosis patients showed significant non-synonymous mutations. Among the drug-resistant TB patients, seven unique significant mutations out of 73 isolates (9.6%) were distributed among Isoniazid-resistant tuberculosis and Multi-Drug Resistant Tuberculosis isolates. No association was found between the mutations and the clinical characteristics of the subjects harboring these isolates.

Conclusion: This study estimated seven unique mutations in drug-resistant tuberculosis and none in drug-sensitive tuberculosis. Isolates harboring was not significantly associated with the participant's treatment outcome and other clinical characteristics. The pyrazinamide resistance testing by the phenotypic and genotypic methods was found to be in concordance.

1. Introduction

Mycobacterium tuberculosis (MTB) has been studied extensively for genetic mutations that confer resistance to drugs used to treat Tuberculosis (TB). The gene *pncA* is one such gene that encodes for pyrazinamidase (PZAse) and helps in the activation of pyrazinamide (PZA) to its active form pyrazinoic acid (POA). Thus, mutation in this gene is linked to PZA resistance.[1]

The drug pyrazinamide and its resistance are being explored in research as this drug mainly acts on slow-growing and persisting bacteria responsible for the relapse of TB. This sterilizing action of pyrazinamide, along with rifampicin, served as the reason for reducing the duration of the treatment regimen from nine to six months.[2]

The drug pyrazinamide is a part of different TB regimens such as Drug susceptible (DS-TB) Tuberculosis regimen, INH-mono/poly drug-resistant TB regimen, and Bedaquiline containing shorter oral Multi-Drug Resistant (MDR) TB regimen.[3] Apart from all bactericidal first-line drugs, this drug is not screened routinely for resistance in the initial phase of the therapy. This is due to a lack of studies reporting resistance to this drug and its burden in patients with TB, and also lacks

* Corresponding author. Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education & Research, Gorimedu, Puducherry, India.
E-mail address: mirunalini3012@gmail.com (M. R).

https://doi.org/10.1016/j.ijtb.2024.03.006
Received 8 January 2024; Received in revised form 8 March 2024; Accepted 13 March 2024
Available online 26 March 2024

an approved molecular marker for resistance. Thus, the use of this drug without screening for resistance for a usual regular duration remains irrational.

Currently, the WHO recommends Liquid Culture-Drug Susceptibility Test (LC-DST) method to look for resistance in pyrazinamide, which has a drawback of increased incidence of false positive results due to sub-optimal activity of pyrazinamide invitro conditions.[4]

Notably, the proportion of this genotypic resistance to pyrazinamide by the *pncA* mutations was higher among Drug-resistant tuberculosis (DR-TB) isolates, ranging around 40–60%.[5–8] Pyrazinamide resistance and failure to pick the drug resistance in the early part of the treatment regimen play some vital roles in treatment failure.[9] Thus, measures to screen for resistance to pyrazinamide in the early phase of the treatment can significantly help in addressing treatment failure patients among DR TB patients, which was 26% compared to 8.7% among DS-TB patients according to the recent India TB Report 2023.[10]

Though India has a high prevalence of tuberculosis, there is still a lacuna in knowledge regarding mutations in *pncA* and the proportion of mutations among different regimen categories of tuberculosis, such as drug-susceptible and DR-TB, and its association with treatment outcomes.

Hence, in this study, we aimed to address this lacuna by quoting the proportion of *pncA* mutation in drug-susceptible and DR-TB patients. The subjects were also followed up until the end of the regimen to record the treatment outcome associated with the *pncA* mutation profile.

2. Methodology

2.1. Design and setting

This longitudinal observational study was conducted in Pondicherry, Union territory of India. The sample collection was done in the Intermediate Reference Laboratory (IRL), Puducherry, which receives samples from the districts of Pondicherry and Karaikal. Samples of all notified pulmonary tuberculosis patients who have been bacteriologically confirmed with a positive result for *Mycobacterium tuberculosis,* irrespective of their age and gender, were eligible for the study. Samples of extrapulmonary tuberculosis patients and those enrolled in M/XDR-TB patients started on all oral regimens were excluded from this study.

The data from the Electronic Health Record (EHR) was collected hand in hand while processing the samples. Patients were followed up till the completion of the regimen, and data like age, gender, occupational status, and treatment outcome as cured/treatment completed (favorable) and default/failure/death (unfavorable) were entered in the case report form.

3. Study procedure

3.1. Decontamination of samples

The samples were collected from IRL, and the samples were subjected to pre-extraction processing such as decontamination by 4% NALC- NaOH method, and the decontaminated samples were cultured for 14–21 days using BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD, United States) according to the manufacturer's instructions.[11,12]

3.2. DNA extraction, amplification, and gene sequencing

The cultured MGIT samples were transferred to 15 mL centrifuge tubes and centrifuged at 4000 rpm for 5 min. The pellet obtained was used for extraction using the Qiagen DNA Mini Kit. The extracted samples were then subjected to amplification for which 5 µl of DNA product along with 12.5 µl master mix (Sigma Aldrich), 4.5 µl nuclease-free water, and 1.5 µl of each forward and reverse primer of sequence described below were added to get a 25 µl PCR product.

Forward primer: 5′-GGCGTCATGGACCCTATATC-3′

Reverse primer: 5′-CAACAGTTCATCCCGGTTC -3′.

The PCR was run at 94 °C for 5 min for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min, and final extension was run at 72 °C for 10 min. The PCR products were then subjected to post PCR purification using NucleoSpin Gel and PCR Clean-up, Mini kit, Macherey Nagel.

Sequencing was done to this PCR product to analyze the sequence and mutations in the *pncA* gene. The gene sequence was studied for mutations using a sequence from the NCBI portal as a reference sequence using BioEdit software version 7.2.5.

3.3. Phenotypic drug susceptibility testing

Phenotypic DST was done using the Broth microdilution method proposed by Leite et al., for which 96 well plates with U-shaped bottoms were used. The samples were diluted and inoculated with 100 mcg of pyrazinamide as a cut-off to find their phenotypic susceptibility. The plate was incubated at 37 °C for around 21 days to detect the organism's growth.[13]

3.4. Sample size and data analysis

Considering the expected proportion of pyrazinamide resistance as 10% with 5% absolute precision and 95% CI, the sample size is 139. Assuming a 10% attrition, the corrected sample size is estimated to be 154.

The categorical data were expressed as frequency and proportions, and the continuous data, such as Age and BMI, were expressed as mean with standard deviation (SD). The association between *pncA* and clinical characteristics was done by Chi-squared/Fisher's Exact test using IBM SPSS statistics software version 19. A P-value less than 0.05 was considered statistically significant.

3.5. Ethical issues

Ethical clearance was obtained from the Institutional Ethics Committee, JIPMER. The samples obtained were de-identified and anonymized to maintain the confidentiality of the patients.

4. Results

4.1. Baseline characteristics

This study aimed to estimate the proportions of *pncA* mutation in patients on DS-TB and DR-TB regimens and to find its association with treatment outcomes. A total of 154 isolates were taken for study purposes, of which 81 (52.6%) were on the DS-TB regimen, and 73 (47.4%) were under the DR-TB regimen. The distribution of patients based on their baseline characteristics is mentioned below (Table 1).

4.2. Analysis of mutation pattern in the pncA gene

There were 21 (13.6%) isolates with mutations irrespective of drug-resistant patterns, and they were found to be scattered throughout the coding region of *pncA*. There was a total of 11 unique mutations identified across the *pncA* gene, which included synonymous and non-synonymous mutations distributed in both DS-TB and DR-TB with proportions of 8.6 and 19.2%, respectively (Fig. 1). The characteristics of the observed mutations are given in Table 2.

4.3. Association of pncA mutations with clinical characteristics

The association between the *pncA* mutation and clinical characteristics such as type of cases, whether new or previously treated, early

Table 1
Baseline characteristics of TB patients distributed among Drug-Susceptible Tuberculosis (DS-TB) and Drug-Resistant Tuberculosis groups (DR-TB).

Baseline characteristics	DS-TB	DR-TB	Total
	N = 81, n (%)	N = 73, n (%)	N = 154, n (%)
Age (years)[a]	46 (15.6)	47.5 (13.7)	46.7 (14.7)
Gender			
Male	58 (71.6)	56 (76.7)	114 (74)
Female	23 (28.4)	17 (23.3)	40 (26)
BMI (kg/m^2)[a]	18.2 (3.5)	18.2 (3.6)	18.3 (3.5)
BMI category(kg/m^2)			
Underweight (<18.5)	44 (54.3)	44 (60.3)	88 (57.1)
Normal (18.5–22.9)	29 (35.8)	23 (31.5)	52 (33.8)
Overweight & Obese (>23)	8 (9.9)	6 (8.2)	14 (9.1)
Status at TB diagnosis			
New Patients	77 (95.1)	65 (89)	142 (92.2)
Previously treated patients	4 (4.9)	8 (11)	12 (7.8)
Tobacco	17 (20.5)	14 (19.4)	31 (20.1)
Alcohol	14 (17.3)	15 (20.8)	29 (18.8)
HIV	1 (1.2)	1 (1.4)	2 (1.3)
Diabetes Mellitus	26 (32.1)	33 (45.2)	59 (38.3)
Occupation			
Unemployed	17 (21)	17 (23.3)	34 (22.1)
Skilled	4 (4.9)	8 (11)	12 (7.8)
Unskilled	22 (24.7)	13 (17.8)	33 (21.4)
Labored	40 (49.4)	34 (47.9)	75 (48.7)

BMI – Body Mass Index, DS-TB – Drug-Susceptible Tuberculosis, DR-TB – Drug-Resistant Tuberculosis, TB-Tuberculosis, HIV- Human Immunodeficiency Virus.
[a] Expressed as mean (SD).

sputum conversion status, and treatment outcome are given in Table 4, and they showed no significant association.

4.4. Treatment outcome

The three mutations seen in unfavorable treatment outcomes were 80 (T > C), 395 (G > C), and 392 G ins mutations (see Table 3). The mutation at 80 (T > C) was associated with treatment failure.

Type of cases.

Among the 73 subjects, eight had a previous treatment history, and 65 were newly treated. The proportion of mutated isolate was higher among the previously treated patients (12.5%) than the new cases (9.2%).

4.5. Phenotypic resistance of pyrazinamide

Phenotypic resistance to pyrazinamide by broth dilution method revealed the pyrazinamide resistance in 8 samples at 100 μg/mL concentration of pyrazinamide, of which six (75%) had mutations in the *pncA* gene and two in isolates with no *pncA* mutation as given in Table 5.

5. Discussion

This study included 154 isolates with drug-susceptible and drug-resistant strains and was analyzed for the mutations in the *pncA* gene. The occurrences of these mutations and their association with the treatment outcome were done by following up with the patients till the end of their regimen. None of the DS-TB patients showed significant non-synonymous mutations. Among the drug-resistant TB patients, seven unique mutations were distributed among the Hr-TB and MDR-TB patients. No association was found between the mutations and the clinical characteristics of the subjects harbouring these isolates.

In this study, considering only the non-synonymous mutations as significant mutations, only seven out of 21 were observed, distributed among the 73 patients with DR-TB regimens (9.6%). This DR-TB group includes Hr-TB isolates and MDR-TB isolates. In a study conducted by Mvelase et al. in South Africa, 80 rifampicin-resistant isolates were analyzed for *pncA* mutation, which revealed the proportion of mutation to be 7.5%, similar to our study.[14]

Few studies were conducted in countries other than India to identify *pncA* proportions on DR-TB or MDR-TB isolates, which showed proportions around 40–60%, with most studies showing proportions around 50%.[5,7,8,15–17] One study by Li et al. from Chongqing, China, in 465 drug-resistant isolates showed that 74.4% of isolates had 124 different types of mutations throughout the *pncA* gene.[6]

This considerable variation in proportions of *pncA* gene mutations observed in other studies compared to ours can be attributed to the diluted numbers of MDR-TB strains and the small number of mutations captured among them, which was less than expected. There was also a lack of knowledge regarding the proportion of *pncA* among the Indian

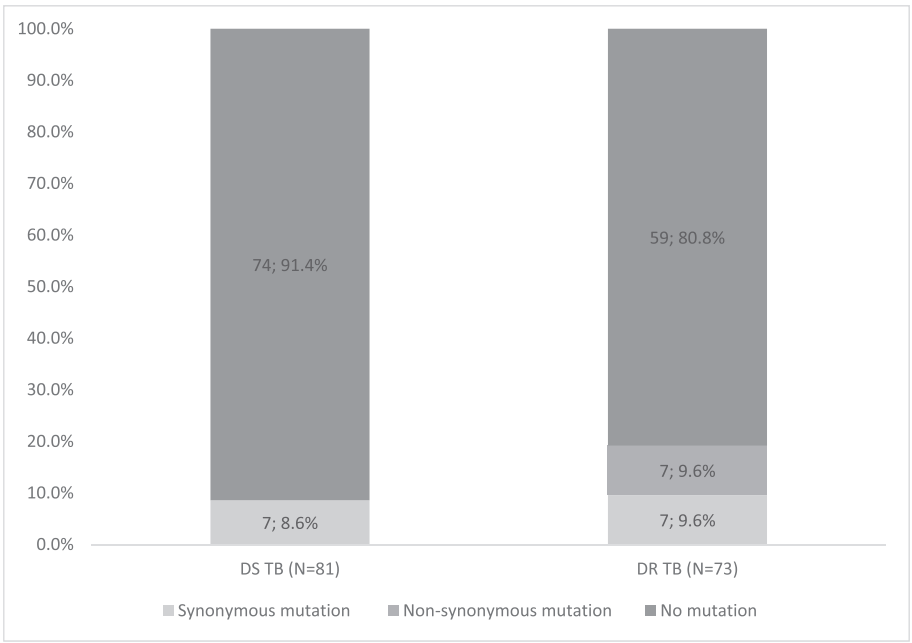


Fig. 1. Proportions of type of mutations among Drug Susceptible tuberculosis (DS-TB) and Drug-Resistant tuberculosis (DR-TB)
The values in the graph are proportions expressed as n; %.

Table 2
Characterization of *pncA* mutations in isolates and their phenotypic drug susceptibility status.

Location of mutation	Nucleotide change	Amino acid change	No of isolates (n = 21)	Phenotypic drug-susceptibility test for pyrazinamide	Drug resistance pattern
Non-synonymous mutations					
80	T > C	Leu27Pro	1	Sensitive	MDR-TB
188	A > G	Asp63Gly	1	Resistant	MDR-TB
226	A > C	Thr76Pro	1	Resistant	Hr-TB
391_392	ins G	Frameshift	1	Resistant	MDR-TB
395	G > C	Gly132Ala	1	Resistant	Hr-TB
395	G > A	Gly132Asp	1	Resistant	Hr-TB
512	C > T	Ala172Val	1	Resistant	MDR-TB
Synonymous mutations					
195	C > T	Synonymous	11	Sensitive	DS-TB, MDR-TB, and Hr-TB
240	C > T	Synonymous	1	Sensitive	Hr-TB
386_395	del ATGTGGTCG	Synonymous	1	Sensitive	DS-TB
420	C > T	Synonymous	1	Sensitive	DS-TB

DS-TB- Drug Susceptible Tuberculosis, MDR-TB- Multi Drug Resistant Tuberculosis, Hr-TB- Isoniazid resistant Tuberculosis.

Table 3
Distribution of *pncA* mutation among different categories of the DR-TB group.

Mutation status		Hr-TB N = 37	MDR-TB N = 36	P-value[a]
pncA mutations	Present **(n = 7)**	2 (5.4)	5 (13.9)	0.261
	Absent **(n = 66)**	35 (94.6)	31 (86.1)	

Hr-TB-Isoniazid resistant TB; MDR TB- Multidrug-resistant TB.
All values are proportions and are expressed as n (%).
[a] Fisher's Exact test.

Table 4
Association of *pncA* mutation with clinical characteristics in drug-resistant tuberculosis patients.

Parameters	*pncA* mutation present n (%)	*pncA* mutation absent n (%)	P-value[a]
Treatment outcome			
Unfavorable (N = 18)	3 (16.7)	15 (83.3)	0.353
Favorable (N = 55)	4 (7.3)	51 (92.7)	
Early sputum conversion status			
Yes (N = 61)	5 (8.2)	56 (91.8)	0.323
No (N = 12)	2 (16.7)	10 (83.3)	
Type of case			
Previously treated patients (N = 8)	1 (12.5)	7 (87.5)	0.573
New Patients (N = 65)	6 (9.2)	59 (90.8)	

All values are proportions expressed as n (%).
[a] Fisher's Exact test.

Table 5
Phenotypic and genotypic mutation status.

Pyrazinamide susceptibility pattern		Phenotypically Resistant (n = 8)	Phenotypically Sensitive (n = 65)	P-value[a]
pncA mutation	Present (n = 7)	6 (75)	1 (1.5)	<0.001
	Absent (n = 66)	2 (25)	64 (98.5)	

All values are proportions, expressed as n (%).
[a] Fisher,s Exact test.

population, which could have been a valid comparator for our study. Another factor is the exclusion of all oral M/XDR-TB regimen that requires 18–21 months of therapy, which was beyond the duration of this study.

Isoniazid-resistance MTB isolates showed a mutation in 5.4% (2 out of 37 isolates) in our study, similar to a study conducted in Hong Kong by Kevin et al. where it was 4.5% (7 out of 157 isolates).[14] Another study in Vietnam showed the proportions as 14.9%, which was relatively higher than ours.[18]

This increased proportion of mutations in previously treated patients is worth considering, as the mutation in *pncA* per se would have led to the recurrence of TB in this group of patients. The above hypothesis is substantiated by the fact that pyrazinamide is active in killing the semi-dormant and persisting bacteria responsible for tuberculosis recurrence.[19] Hence, in patients with relapse of tuberculosis, screening for pyrazinamide resistance might help to achieve a favorable outcome of tuberculosis and prevent further episodes.

The proportion of *pncA* mutations among unfavorable treatment outcomes was 16.7% compared to 7.3% among the favorable treatment group. A study conducted in Tanzania, USA, showed that among the patients who had failed in treatment, 83% had pyrazinamide resistance compared to only 41.6% in patients with successful treatment.[20] A study conducted in South India on treatment failure patients concluded that 78% (39 out of 50) of patients showed a mutation in *pncA*.[21]

Though hypothesizing that mutation affects the treatment outcome, we could find four mutations in patients with favorable treatment outcomes in this study. This discrepancy of having a favorable treatment outcome despite having *pncA* mutation can be due to the effect of other drugs in the regimen.

Further, to discuss the individual significant mutations, they mainly were point substitution mutations (six). The mutation observed in position 226 (A > C) led to the change of amino acid (Threonine to Proline), which was observed in a Pakistan study.[22] Another study from China also reported that the same mutation also showed reduced activity of the pyrazinamidase.[5]

Similarly, there was a substitution mutation in position 188 (A > G) and 395 (G > A), leading to a change in amino acid Aspartate to Glycine and Glycine to Aspartate, respectively. These mutations were reported in a study in China by Li et al., which showed the presence of these mutations.[6]

The other mutations, 80(T > C), 395(G > C), 512(C > T), and 392 (ins G), were reported in the WHO catalog of 2021, where they have listed mutations in *Mycobacterium tuberculosis* complex and their association with drug-resistance.[23]

Among the 73 drug-resistant isolates, 70 showed concordant results with genotypic and phenotypic susceptibility testing. Two isolates did not have any mutation but showed phenotypic resistance. Similarly, in other studies, isolates showed resistance to phenotypic DST but lacked the mutation in the *pncA* gene.[5] This can be due to false positive results, which can be due to factors such as acidic pH and inoculum size influencing the action of pyrazinamide.[9,24,25]

The main strength of this study lies in the study design, where characterizing the mutation goes on one side, following up with the patients for the outcome of their treatment, and associating the mutations with treatment outcomes, which helps us to find the clinical burden of this mutation in treating the patients.

This study's main limitation was the fewer mutations observed due

to a smaller number of MDR-TB strains. As only DR-TB subjects were considered for analysis, numbers were not powered enough to attain a conclusion. To overcome these challenges, studies with larger sample sizes focussing mainly on M/XDR-TB subjects or treatment failure subjects may be considered.

Another limitation was detecting pyrazinamide phenotypic susceptibility using the broth dilution method, where WHO recommends LC-DST for phenotypic susceptibility testing of drugs. However, our study did not consider this due to the limited resources and time.

By focussing on mutational studies among the treatment failure group of subjects, we can impact tuberculosis management. With considerable research data on specific mutations, this gene can be used as a marker to predict the diagnosis of resistance among patients and to coin out the most appropriate regimen for pyrazinamide-resistant subjects.

This study identified the proportions of *pncA* mutation in DR-TB as 9.6%, and no significant mutation was seen among DS-TB isolates. The mutations in the *pncA* gene were not associated with the clinical characteristics. The pyrazinamide resistance testing by the phenotypic and genotypic methods was found to be in concordance. More future studies with larger sample sizes and focussing mainly on drug-resistant and treatment failure isolates might help in including *pncA* mutation as a marker for early detection of pyrazinamide resistance.

Funding

JIPMER intramural fund, JIPMER, Puducherry, India.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Via LE, Savic R, Weiner DM, et al. Host-mediated bioactivation of pyrazinamide: implications for efficacy, resistance, and therapeutic alternatives. *ACS Infect Dis [Internet]*. 2015;1:203–214. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4467917/. Accessed June 29, 2023.
2. Gopal P, Grüber G, Dartois V, Dick T. Pharmacological and molecular mechanisms behind the sterilizing activity of pyrazinamide. *Trends Pharmacol Sci*. 2019;40:930–940.
3. Guidelines for PMDT in India.pdf [Internet]. [cited 2023 May 18]. Available from: https://tbcindia.gov.in/WriteReadData/l892s/8368587497Guidelines%20for%20PMDT%20in%20India.pdf.
4. WHO consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection, 2021 update. Available from: https://www.who.int/publications-detail-redirect/9789240029415. Accessed June 21, 2023.
5. Shi D, Zhou Q, Xu S, Zhu Y, Li H, Xu Y. Pyrazinamide resistance and pncA mutation profiles in multi-drug resistant Mycobacterium. *Tuberculosis. Infect Drug Resist*. 2022;15:4985–4994.
6. Li K, Yang Z, Gu J, Luo M, Deng J, Chen Y. Characterization of pncA mutations and prediction of PZA resistance in Mycobacterium tuberculosis clinical isolates from chongqing, China. *Front Microbiol [Internet]*. 2021;11, 594171. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7832174/. Accessed July 5, 2023.
7. Shrestha D, Maharjan B, Thapa J, et al. Detection of mutations in pncA in Mycobacterium tuberculosis clinical isolates from Nepal in association with pyrazinamide resistance. *Curr Issues Mol Biol [Internet]*. 2022;44:4132–4141. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9497661/. Accessed July 3, 2023.
8. Ei PW, Mon AS, Htwe MM, et al. Pyrazinamide resistance and pncA mutations in drug resistant Mycobacterium tuberculosis clinical isolates from Myanmar. *Tuberc Edinb Scotl*. 2020;125, 102013.
9. Siddiqi S, Ahmed A, Asif S, et al. Direct drug susceptibility testing of Mycobacterium tuberculosis for rapid detection of multi-drug resistance using the bactec MGIT 960 system: a multicenter study. *J Clin Microbiol [Internet]*. 2012;50:435–440. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3264138/. Accessed July 3, 2023.
10. TB Annual report-2023_23- 03-2023_LRP.pdf. Available from: https://tbcindia.gov.in/WriteReadData/l892s/5646719104TB%20AR-2023_23-%2003-2023_LRP.pdf. Accessed July 7, 2023.
11. Bradner L, Robbe-Austerman S, Beitz DC, Stabel JR. Chemical decontamination with N-Acetyl-l-Cysteine–Sodium hydroxide improves recovery of viable Mycobacterium avium subsp. paratuberculosis organisms from cultured milk. *J Clin Microbiol [Internet]*. 2013;51:2139–2146. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3697694/. Accessed October 8, 2023.
12. Krüüner A, Yates MD, Drobniewski FA. Evaluation of MGIT 960-based antimicrobial testing and determination of critical concentrations of first- and second-line antimicrobial drugs with drug-resistant clinical strains of Mycobacterium tuberculosis. *J Clin Microbiol*. 2006;44:811–818.
13. Leite CQ, Beretta AL, Anno IS, Telles MA. Standardization of broth microdilution method for Mycobacterium tuberculosis. *Mem Inst Oswaldo Cruz*. 2000;95:127–129.
14. Ng KKM, Yip PCW, Leung PKL. Prevalences of levofloxacin resistance and pncA mutation in isoniazid-resistant Mycobacterium tuberculosis in Hong Kong. *Hong Kong Med J Xianggang Yi Xue Za Zhi*. 2021;27:421–427.
15. Che Y, Bo D, Lin X, Chen T, He T, Lin Y. Phenotypic and molecular characterization of pyrazinamide resistance among multidrug-resistant Mycobacterium tuberculosis isolates in Ningbo, China. *BMC Infect Dis [Internet]*. 2021;21:605. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8228925/. Accessed July 1, 2023.
16. Liu Q, Yang D, Qiu B, et al. Drug resistance gene mutations and treatment outcomes in MDR-TB: a prospective study in Eastern China [Internet] *PLoS Negl Trop Dis*. 2021;15, e0009068. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7850501/. Accessed July 3, 2023.
17. Sodja E, Koren S, Toplak N, Truden S, Žolnir-Dovč M. Next-generation sequencing to characterise pyrazinamide resistance in Mycobacterium tuberculosis isolates from two Balkan countries. *J Glob Antimicrob Resist [Internet]*. 2022;29:507–512. Available from: https://www.sciencedirect.com/science/article/pii/S221371652100254X. Accessed July 5, 2023.
18. Huy NQ, Lucie C, Hoa TTT, et al. Molecular analysis of pyrazinamide resistance in Mycobacterium tuberculosis in Vietnam highlights the high rate of pyrazinamide resistance-associated mutations in clinical isolates. *Emerg Microbes Infect*. 2017;6:e86.
19. Zhang Y, Scorpio A, Nikaido H, Sun Z. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of Mycobacterium tuberculosis to pyrazinamide. *J Bacteriol*. 1999;181:2044–2049.
20. Juma SP, Maro A, Pholwat S, et al. Underestimated pyrazinamide resistance may compromise outcomes of pyrazinamide containing regimens for treatment of drug susceptible and multi-drug-resistant tuberculosis in Tanzania. *BMC Infect Dis*. 2019;19:129.
21. Muthaiah M, Jagadeesan S, Ayalusamy N, et al. Molecular epidemiological study of pyrazinamide-resistance in clinical isolates of Mycobacterium tuberculosis from South India. *Int J Mol Sci [Internet]*. 2010;11:2670–2680. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2920559/. Accessed July 3, 2023.
22. khan MT, Malik SI, Ali S, et al. Pyrazinamide resistance and mutations in pncA among isolates of Mycobacterium tuberculosis from Khyber Pakhtunkhwa, Pakistan. *BMC Infect Dis [Internet]*. 2019;19:116. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6364397/. Accessed July 1, 2023.
23. Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance. Available from: https://www.who.int/publications-detail-redirect/9789240028173. Accessed July 6, 2023.
24. Chedore P, Bertucci L, Wolfe J, Sharma M, Jamieson F. Potential for erroneous results indicating resistance when using the Bactec MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to pyrazinamide. *J Clin Microbiol*. 2010;48:300–301.
25. Piersimoni C, Mustazzolu A, Giannoni F, Bornigia S, Gherardi G, Fattorini L. Prevention of false resistance results obtained in testing the susceptibility of Mycobacterium tuberculosis to pyrazinamide with the bactec MGIT 960 system using a reduced inoculum. *J Clin Microbiol [Internet]*. 2013;51:291–294. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3536208/. Accessed July 3, 2023.

Indian Journal of Tuberculosis 71 (2024) S86–S90

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Long-term follow-up of contacts of drug-resistant tuberculosis cases in high-burden areas of Mumbai, India

Yatin Dholakia *, Laxmi Govekar, Nerges Mistry

The Foundation for Medical Research, India

ARTICLE INFO

Keywords:
DRTB contacts
IGRA
Vitamin D
Development of active TB disease
LTBI

ABSTRACT

Background: Drug-resistant tuberculosis (DRTB) is a significant public health threat particularly in high burden areas like Mumbai, India. Contacts of DRTB cases are highly vulnerable to infection and development of active disease. In this study we assess long-term outcomes of contacts of DRTB cases, focusing on active TB development and the potential role of IGRA, vitamin D status and supplementation.

Methods: A cohort of 262 DRTB contacts identified from a prior case-control study conducted in Mumbai were enlisted for the study. Interviews were conducted, and data were analysed using descriptive statistics and logistic regression.

Results: Of the 262 contacts, 34.73% had LTBI. Three contacts (1.36%) developed active TB, with a crude incidence rate of 4.64 per 1000 people. Vitamin D deficiency was prevalent in 75.3% of contacts, and all three TB cases were vitamin D deficient. Vitamin D supplementation showed a non-significant trend in reducing TB risk (OR = 0.56, p = 0.492). IGRA status did not significantly predict TB development.

Conclusion: This study provides valuable insights into the long-term outcomes of contacts of DRTB cases. While baseline IGRA did not prove to predict development of active TB, association between vitamin D deficiency and TB development highlights the need for larger studies and development of more effective screening tools. The study contributes valuable information to TB control strategies in high-burden areas.

1. Introduction

Drug resistant tuberculosis continues to be a major global public health risk threatening to reverse the efforts to END TB by 2035.[1,2] An estimated 450,000 MDR/RR TB cases were reported globally in the year 2021. This was an increase in the number of cases by 3.1% as compared to that of 2020 as a result of the COVID-19 pandemic.[3] In 2021, only about one in three patients with drug resistant tuberculosis accessed treatment, with 191,000 reported deaths; twice the number of deaths everyday as compared to COVID-19 deaths.[3,4]

In India, 48,232 MDR/RR-TB, 8455 Pre-XDR-TB and 376 XDR-TB patients were diagnosed in the year 2021, of which 90%, 89% and 89% accessed the treatment for tuberculosis respectively.[5] Contacts of tuberculosis patients are a high-risk vulnerable group for developing infection and progression to disease.[6,7] Findings from a meta-analysis on studies carried out in low- and middle-income countries suggests that the prevalence of TB among DRTB contacts was 3.4%. This was also associated with majority of contacts developing active TB disease within one year after exposure.[8,9]

Interferon Gamma Release Assay (IGRA) has proven to be a valuable tool in identifying latent TB infections, aiding in the timely initiation of preventive measures and treatment, ultimately contributing to the control and prevention of TB transmission. The sensitivity and specificity of IGRA in detecting tuberculosis (TB) infection can vary based on factors such as the population being tested, the prevalence of TB, and the presence of other conditions that might affect the immune response. Both TST and IGRA have low ability to predict development of active TB, additionally several factors such as impaired immune response and technical issues also affect the performance of these tests.[10–12]

Role of vitamin D in tuberculosis has been studied widely due to its effect on both innate and acquired immunity as well as its anti-inflammatory action.[13] In-vitro studies have shown that vitamin D inhibits the growth of *Mycobacterium tuberculosis* through these processes.[14–16] Huang et al. in their meta-analysis revealed that an association between vitamin D deficiency and tuberculosis was more likely a risk factor rather than the consequence of TB infection.[17]

In this retrospective cohort study, contacts of DRTB patients were followed-up to assess the development of active TB and the role of

* Corresponding author. The Foundation for Medical Research, Dr. Kantilal J. Sheth Memorial Building 84A, R.G. Thadani Marg, Worli, Mumbai, 400 018, India.
E-mail addresses: yatindholakia@gmail.com (Y. Dholakia), laxmigovekar@gmail.com (L. Govekar), fmr@fmrindia.org (N. Mistry).

https://doi.org/10.1016/j.ijtb.2024.04.002
Received 16 February 2024; Received in revised form 31 March 2024; Accepted 2 April 2024
Available online 2 April 2024

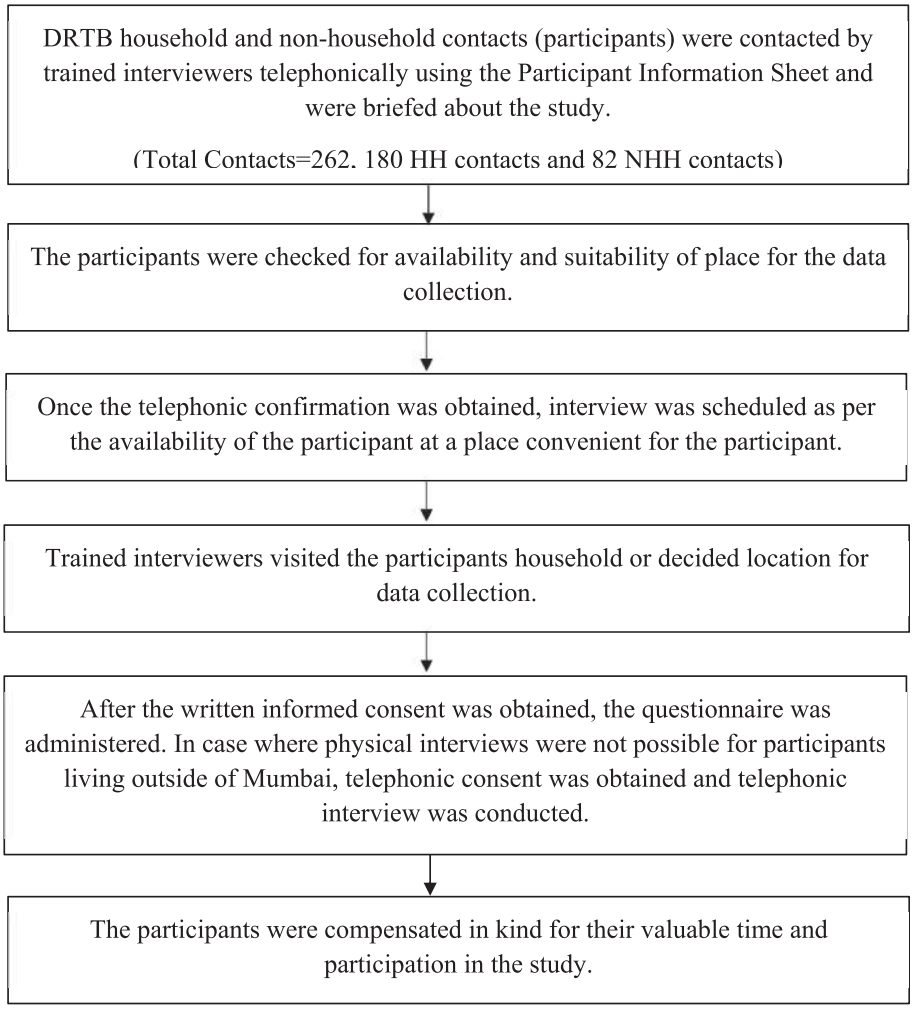


Fig. 1. Work flow.

baseline IGRA, vitamin D status and vitamin D supplementation.

2. Methodology

A case-control study of Vitamin D status and adult drug resistant tuberculosis was conducted in three high DRTB burden wards in Mumbai, India between January 2020 and December 2020.[18] The case-control study was conducted among patients with drug resistant tuberculosis (DRTB), household contacts and non-household contact 18–60 years of age group in M-east, M-west and H-east wards of Mumbai. Household contacts were recruited in 1:2 ratio whereas non-household contacts were recruited in 1:1 ratio of cases and controls. As a part of that study, contacts of DRTB patients had been screened for active tuberculosis through symptom screening, chest X-ray examination and sputum testing by CBNAAT; Interferon Gamma Release Assay (IGRA) testing for latent TB infection and estimation of serum vitamin D levels. Participants with positive IGRA tests were not provided tuberculosis preventive treatment (TPT) as there were no existing guidelines during the study period. Individuals with severe and moderate levels of vitamin D deficiency were provided with supplemental Vit D3 60,000 IU weekly for 8 and 12 weeks respectively as per the clinical guidelines. Detailed results of the study have been published.[18]

The present retrospective cohort study involved household and non-household contacts of DRTB patients which were enrolled in the Vitamin D-MDR TB Study.[18] These individuals were contacted by the trained interviewers using participant information sheets briefing them about the study. After obtaining telephonic consent, interviews were conducted at a place convenient to the interviewee to ensure privacy after obtaining written informed consent (Fig. 1).

A structured questionnaire was used for the interview. The questionnaire had two sections-i) Past clinical history during the two years; ii) Current clinical symptoms and investigation details. We also asked whether any other family member had been diagnosed with active TB during the last two years or were symptomatic at the time of interview.

In case of symptomatic individuals, chest X-ray and sputum tests were carried out as per guidelines and results recorded. Participants were compensated in kind after the interview.

2.1. Data analysis

Data was recorded on MS Excel sheet and was exported into IBM SPSS Statistics v25 for the analysis. Descriptive statistics was carried out to describe proportions with the recorded variables. Association between variables was carried out using logistic regression.

2.2. Ethical approval and permissions

The study was approved by the Institutional Ethics Committee (FMR/IREC/TB/01/2023). All participants enrolled in the study provided their informed consent for inclusion before their participation in the study.

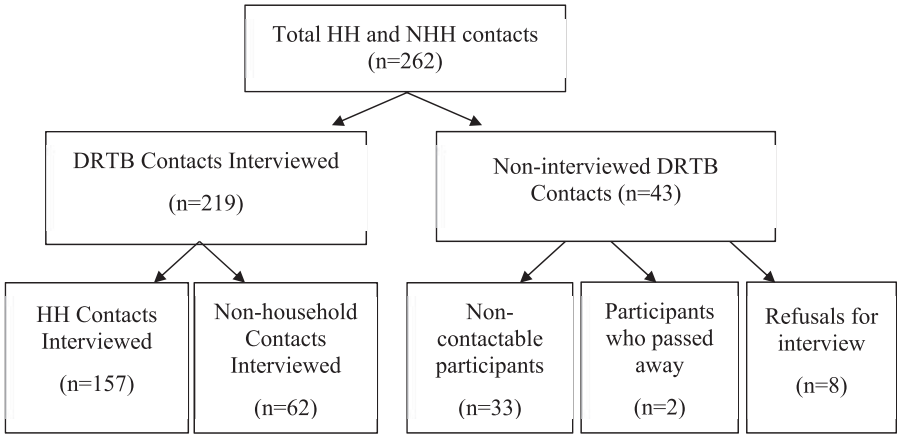


Fig. 2. Recruitment flowchart.

Table 1
Demographic, baseline clinical and follow-up proportion details.

Characteristics		Household Contacts	Non-Household Contacts	Total
		n (%)	n (%)	n (%)
Gender distribution	Male	73 (46.5%)	25 (40.3%)	98 (44.75%)
	Female	84 (53.5%)	37 (59.7%)	121 (55.25%)
Place of Interview	Elsewhere	12 (7.6%)	24 (38.7%)	36 (16.44%)
	Home	135 (86%)	35 (56.5%)	170 (77.62%)
	Telephonic	10 (6.4%)	3 (4.8%)	13 (5.94%)
Occupation (pulled from the previous study)	Unemployed	6 (3.8%)	4 (6.5%)	10 (4.6%)
	Homemaker	57 (36.3%)	20 (32.3%)	77 (35.2%)
	Student	13 (8.3%)	9 (14.5%)	22 (10%)
	Daily wage worker/casual labourer	19 (12.1%)	2 (3.2%)	21 (9.6%)
	Salaried	40 (25.5%)	23 (37.1%)	63 (28.8%)
	Self-employed	21 (13.4%)	4 (6.5%)	25 (11.4%)
	Retired	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Other	1 (0.6%)	0 (0.0%)	1 (0.5%)
Baseline IGRA status	Negative	101 (64.3%)	42 (67.7%)	143 (65.30%)
	Positive	56 (35.7%)	20 (32.3%)	76 (34.70%)
Baseline Vitamin D status	Optimal level	42 (26.8%)	12 (19.4%)	54 (24.7%)
	Mild-moderate deficiency	70 (44.6%)	32 (51.6%)	102 (46.6%)
	Severe deficiency	45 (28.7%)	18 (29%)	63 (28.8%)
Vitamin D Supplementation	Provided	95 (60.5%)	2 (3.2%)	97 (44.3%)
	Not provided	20 (12.7%)	50 (80.6%)	70 (32%)
	Not required (Optimal levels)	42 (26.8%)	10 (16.1%)	52 (23.7%)
Baseline X-ray findings	Abnormal	27 (17.2%)	8 (12.9%)	35 (15.98%)
	Normal	130 (82.8%)	54 (87.1%)	184 (84.02%)
History of TB	Tuberculosis	2 (1.3%)	1 (1.6%)	3 (1.4%)
Clinical history during past two years	HIV	0 (0%)	0 (0%)	0 (0%)
	Diabetes	13 (8.3%)	4 (6.5%)	17 (7.7%)
	COVID	1 (0.6%)	3 (4.8%)	4 (1.8%)
	Blood pressure	18 (11.5%)	6 (9.7%)	24 (10.95%)
	Other diseases	7 (4.5%)	4 (6.45%)	11 (5.0%)
Any household members identified with TB	No	155 (98.7%)	59 (95.2%)	214 (97.7%)
	Yes	2 (1.3%)	3 (4.8%)	5 (2.3%)
Current Substance Abuse	Only smoke cigarettes	4 (2.5%)	2 (3.2%)	6 (2.74%)
	Only smoke bidis	2 (1.3%)	0 (0.0%)	2 (0.91%)
	Only drink alcohol or beer	0 (0.0%)	2 (3.2%)	2 (0.91%)
	Other addictive substances	27 (17.2%)	5 (8.1%)	32 (14.61%)
	More than one substance abuse mentioned	9 (5.7%)	4 (6.45%)	13 (5.94%)
	No substance abuse	115 (73.2%)	49 (79%)	164 (74.89%)
Miscellaneous	Experienced weight loss in the past 1 month	8 (5.1%)	4 (6.5%)	12 (5.48%)
	Admitted to a hospital for any illness in the past 1 month	3 (1.9%)	0 (0%)	3 (1.37%)

3. Results

A total of 262 DRTB contacts (180 household and 82 non-household) were identified from the previous case-control study. Of these, 219 contacts were interviewed (Fig. 2). The details on demography, vitamin D levels, IGRA and chest X-ray are shown in Table 1. Total person year follow up was 645.7 with an average of 2.95 years for all contacts.

In 262 contacts, LTBI was documented in 91 contacts (34.73%) and in 76 contacts of total 219 (34.70%) that were included for analysis.

Over all 3 contacts were identified as having developed active pulmonary TB during the period; of which 2 household contacts developed DRTB (1 MDR-TB and 1 XDR-TB) and 1 non-household contact developed DSTB. Crude Incidence rate[19] (CIR) was calculated using the formula: *number of new cases divided by total person-time at risk*, which is

Table 2
Binomial logistic regression of predictor variables analysed for the development of tuberculosis.

Predictor variables for development of tuberculosis	B	S.E.	Wald	df	Sig.	Exp(B)
Age	−0.034	0.053	0.409	1	0.523	0.967
Occupation	0.16	0.364	0.193	1	0.661	1.173
Baseline BMI	−0.455	0.243	3.492	1	0.062	0.635
Type of contact	0.239	1.234	0.038	1	0.846	1.27
Gender	0.489	1.232	0.157	1	0.692	1.63
IGRA Status	−0.062	1.233	0.003	1	0.96	0.94
Level of IGRA positivity	−0.352	0.908	0.15	1	0.699	0.704
Vitamin D levels	−0.582	0.847	0.472	1	0.492	0.559
Vitamin D supplementation	−0.945	0.99	0.912	1	0.34	0.389
X-ray Status	−0.984	1.239	0.631	1	0.427	0.374
Comorbidities:						
COVID	−17.91	18463.175	0	1	0.999	0
Diabetes	2.559	1.284	3.974	1	0.046	12.929
Hypertension	−18.14	7564.936	0	1	0.998	0

often expressed per 1000 or 100,000 people. The incidence rate for all contacts was 4.64 per 1000 people (4.24 and 5.76 per 1000 people for household contacts and non-household contacts, respectively).

During the interviews, 5 other household contacts were reported to develop active TB (2 HH and 3NHH) among the families of DRTB contacts originally studied.

The mean time to develop TB among the three contacts who developed TB was 386 days (12.7 months) which was calculated as the difference in dates of the baseline clinical assessment and the initiation date of TB treatment.

The yield of TB disease identified was 1.36%, whereas the number needed to screen (NNS) which is reciprocal of the yield was 72.99, indicating 73 contacts needs to be screened to find one positive case.

Of the initial 76/219 IGRA positive contacts, one household contact developed active TB within 2.95 years whereas among the initial 143/219 IGRA negative contacts, two developed active TB. The OR of 0.94 and did not show statistical significance ($p = 0.96$).

Vitamin D deficiency was observed in 165 of 219 DRTB contacts at baseline. All three contacts who developed TB were vitamin D deficient. One contact who developed TB had been supplemented with vitamin D for 8 weeks; retested vitamin D levels were found to be optimal. This contact developed TB two and half years post supplementation. The odds ratio for vitamin D levels and development of tuberculosis was 0.56 indicating that, for each unit increase in levels of vitamin D, the odds of developing TB decrease by about 44.1%. However, this effect ($p = 0.492$) was not statistically significant.

All three contacts who developed active TB had low levels of vitamin D at baseline. Among 76 IGRA positive contacts, 58 had vitamin D deficiency, of whom 38 were supplemented with vitamin D for 8 or 12 weeks based on level of deficiency.

We analysed the variables age, gender, type of contact, baseline IGRA status, level of IGRA positivity, occupation, comorbidities, baseline vitamin D levels, vitamin D supplementation, baseline X-ray status and substance abuse as predictors for the development of tuberculosis and found no statistically significant effect on development of TB (Table 2).

4. Discussion

Drug-resistant tuberculosis (DRTB) is a significant public health threat and likely to thwart efforts to eliminate tuberculosis by 2035. Our study's findings highlight the challenges of management of contacts of DRTB cases. The study's focus on contacts of DRTB cases in Mumbai, India, provides valuable information to understanding of TB transmission and the challenges faced in high-burden regions.

Our finding of a 34.73% prevalence of LTBI among contacts of DRTB cases compares with a meta-analysis on low- and middle-income countries,[20] with a 3.4% prevalence of TB among DRTB contacts. This high prevalence of LTBI in DRTB contacts emphasizes the vulnerability of DRTB contacts and the need for effective preventive measures.

We had an incidence rate of 4.64 per 1000 people among our cohort. This, in comparison with similar studies, such as a systematic review in Tajikistan[21] which reported a lower incidence, suggests regional variations in transmission dynamics and also provides valuable information on the risk of active TB among DRTB contacts. The study's reliance on chest X-ray, serum vitamin D levels, and IGRA for screening is in line with recommended methods. Lack of significance in IGRA results highlights the limitations of current screening tools in predicting active TB.

The association between vitamin D deficiency and active TB development, as seen in this study, has been studied extensively.[17] This being so, our findings diverge from some prior research.[22,23] Vitamin D supplementation did not have a significant role in reducing TB risk, this needs to be explored further in larger clinical trials.

To achieve TB elimination goals, contacts of DRTB cases who are at risk of infection and developing TB need to be screened periodically. Screening helps in identifying incident TB disease and LTBI who can then be offered appropriate care. The two tests used to identify LTBI: TST and IGRA are not specific and predictive of development of active TB disease.[24–26]

Our study had some limitations. We could not repeat IGRA at the time of interview due to resource limitation. We missed out on the number of contacts who would have turned positive or reverted from their positive status. However, as those who developed active TB did so within the first year and this would not have significantly affected our findings. Similarly, we could not assess Vitamin D levels.

Another limitation of the study was the absence of genotypic data for both the DRTB cases and the contacts who developed active TB which could have provided insights into the transmissibility patterns of DRTB within close contacts. Furthermore, the observation that household contacts of DRTB cases developed DRTB while non-household contacts predominantly had drug-susceptible TB underscores the potential importance of household transmission in DRTB dissemination. However, without genotypic data, it is not possible to definitively attribute these differences to household transmission dynamics.

5. Conclusions

To achieve TB elimination, there is a need to identify individuals at risk for developing active TB. High risk contacts and vulnerable populations need to be screened and assessed for their vulnerability to developing active TB and initiating appropriate TPT. We did not find IGRA as a fool proof test to identify such individuals, although we did see Vitamin D deficiency associated with TBD development. Tests that can unequivocally identify individuals at risk for developing active TBD are the need of the hour.

Finally, our study on contacts of DRTB cases in Mumbai, India, provides crucial insights into the challenges faced in high-burden areas, highlights the complex dynamics of TB transmission, the limitations of existing screening methods, and the need for continued research to inform targeted and effective TB control strategies.

Author contribution

YD conceptualised the study; YD, NM, LG designed the study and tools; LG did the data collection and analysis; All authors wrote the manuscript including the final version.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The study was supported by a grant from The Maharashtra State Anti TB Association.

The authors are thankful to the field researchers Nilesh Shahasane and Shazia Shaikh for data collection in the field. We acknowledge the help of Syna Mody intern FMR in the analysis. Finally, we thank all respondents for their cooperation.

References

1. Global Tuberculosis Programme, World Health Organization. *The End TB Strategy*; 2015. https://www.who.int/publications/i/item/WHO-HTM-TB-2015.19.
2. Pai M. The End TB Strategy: India can blaze the trail. *Indian J Med Res*. 2015;141: 259–262.
3. Drug-resistant TB. Accessed at https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022/tb-disease-burden/2-3-drug-resistant-tb.
4. Bagcchi S. WHO's global tuberculosis report 2022. *Lancet Microbe*. 2023;4:e20.
5. India TB report. Accessed at https://tbcindia.gov.in/WriteReadData/IndiaTBReport2022/TBAnnaulReport2022.pdf; 2022.
6. Reichler MR, Khan A, Sterling TR, et al. Risk and timing of tuberculosis among close contacts of persons with infectious tuberculosis. *J Infect Dis*. 2018;218(6): 1000–1008. https://doi.org/10.1093/infdis/jiy265.
7. Pinto PFPS, Teixeira CSS, Ichihara MY, et al. Incidence and risk factors of tuberculosis among 420 854 household contacts of patients with tuberculosis in the 100 Million Brazilian Cohort (2004-18): a cohort study. *Lancet Infect Dis*. 2024;24 (1), 46-56. 78.
8. Contact investigation for tuberculosis: a systematic review and meta-analysis, Fox Gregory J, Barry Simone E, Britton Warwick J, Marks Guy B. *Eur Respir J*. 2013; 41:140–156. https://doi.org/10.1183/13993003.50708-2012. *Eur Respir J*. 2015;46 (2):578.
9. Shah NS, Yuen CM, Heo M, Tolman AW, Becerra MC. Yield of contact investigations in households of patients with drug-resistant tuberculosis: systematic review and meta-analysis. *Clin Infect Dis*. 2014;58(3):381–391. https://doi.org/10.1093/cid/cit643.
10. Carranza C, Pedraza-Sanchez S, de Oyarzabal-Mendez E, Torres M. Diagnosis for latent tuberculosis infection: new alternatives. *Front Immunol*. 2020;11:2006. https://doi.org/10.3389/fimmu.2020.02006. Published 2020 Sep. 10.
11. Yang Y, Wang H-J, Hu W-L, Bai G-N, Hua C-Z. Diagnostic value of interferon-gamma release assays for tuberculosis in the immunocompromised population. *Diagnostics*. 2022;12(2):453.
12. Goletti D, Delogu G, Matteelli A, Migliori GB. The role of IGRA in the diagnosis of tuberculosis infection, differentiating from active tuberculosis, and decision making for initiating treatment or preventive therapy of tuberculosis infection. *Int J Infect Dis*. 2022;124(Suppl 1):S12–S19. https://doi.org/10.1016/j.ijid.2022.02.047.
13. Papagni R, Pellegrino C, Di Gennaro F, et al. Impact of vitamin D in prophylaxis and treatment in tuberculosis patients. *Int J Mol Sci*. 2022;23(7):3860. https://doi.org/10.3390/ijms23073860. Published 2022 Mar 31.
14. Rockett KA, Brookes R, Udalova I, Vidal V, Hill AV, Kwiatkowski D. 1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of Mycobacterium tuberculosis in a human macrophage-like cell line. *Infect Immun*. 1998;66(11):5314–5321. https://doi.org/10.1128/IAI.66.11.5314-5321.199815.
15. Coussens A, Timms PM, Boucher BJ, et al. 1alpha,25-dihydroxyvitamin D3 inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. *Immunology*. 2009;127(4):539–548. https://doi.org/10.1111/j.1365-2567.2008.03024.x16.
16. Brighenti S, Bergman P, Martineau AR. Vitamin D and tuberculosis: where next? *J Intern Med*. 2018. https://doi.org/10.1111/joim.12777. Published online May 27.
17. Huang SJ, Wang XH, Liu ZD, et al. Vitamin D deficiency and the risk of tuberculosis: a meta-analysis. *Drug Des Devel Ther*. 2016;11:91–102. https://doi.org/10.2147/DDDT.S79870. Published 2016 Dec 28.
18. Shukla A, Bromage S, Dholakia Y, et al. Case-control study of vitamin D status and adult multidrug-resistant pulmonary TB. *Int J Tuberc Lung Dis*. 2022;26(9):826–834. https://doi.org/10.5588/ijtld.21.0639.
19. Tenny S, Boktor SW. Incidence. In: *StatPearls. Treasure Island (FL)*. StatPearls Publishing; 2024 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430746/.
20. Sagili KD, Muniyandi M, Shringarpure K, et al. Strategies to detect and manage latent tuberculosis infection among household contacts of pulmonary TB patients in high TB burden countries - a systematic review and meta-analysis. *Trop Med Int Health*. 2022;27(10):842–863. https://doi.org/10.1111/tmi.13808.
21. Rekart ML, Aung A, Cullip T, et al. Household drug-resistant TB contact tracing in Tajikistan. *Int J Tuberc Lung Dis*. 2023;27(10):748–753. https://doi.org/10.5588/ijtld.23.0066.
22. Ganmaa D, Khudyakov P, Buyanjargal U, et al. Risk factors for active tuberculosis in 938 QuantiFERON-positive schoolchildren in Mongolia: a community-based cross-sectional study. *BMC Infect Dis*. 2019;19(1):532. https://doi.org/10.1186/s12879-019-4160-7. Published 2019 Jun 17.
23. Patterson B, Smith D, Telford A, et al. Vitamin D deficiency predicts latent TB reactivation independent of preventive therapy: a longitudinal study. *Int J Tuberc Lung Dis*. 2020;24(9):916–921. https://doi.org/10.5588/ijtld.19.0605.
24. Paradkar M, Padmapriyadarsini C, Jain D, et al. Tuberculosis preventive treatment should be considered for all household contacts of pulmonary tuberculosis patients in India [published correction appears in PLoS One. 2020 Dec 31;15(12):e0244914]. *PLoS One*. 2020;15(7), e0236743. https://doi.org/10.1371/journal.pone.0236743. Published 2020 Jul 29.
25. Abubakar I, Drobniewski F, Southern J, et al. Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. *Lancet Infect Dis*. 2018;18(10): 1077–1087. https://doi.org/10.1016/S1473-3099(18)30355-4.
26. van Geuns D, Arts RJW, de Vries G, et al. Screening for tuberculosis infection and effectiveness of preventive treatment among people with HIV in low-incidence settings. *AIDS*. 2024;38(2):193–205. https://doi.org/10.1097/QAD.0000000000003747.

Indian Journal of Tuberculosis 71 (2024) S91–S96

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Isoniazid monoresistant tuberculosis treatment outcomes in Puducherry, South India - A mixed methods study

Arifunhera J [a,1], Mirunalini R [a,2,*], Palanivel Chinnakali [b,2]

[a] Department of Pharmacology, JIPMER, Puducherry, India
[b] Department of Preventive and Social Medicine, JIPMER, Puducherry, India

ARTICLE INFO

Keywords:
6 (H)RZE
Lfx
Adherence
Medication shortage
Turn-around time

ABSTRACT

Setting: On a programmatic basis, a new regimen was introduced in the National Tuberculosis Elimination Programme for isoniazid monoresistant tuberculosis in a few states in India.
Objective: To describe the clinical attributes and treatment outcomes of isoniazid mono-resistant tuberculosis patients on the new 6-month levofloxacin-containing regimen in Puducherry, India.
Design: The study is designed as a convergent parallel mixed-methods study: a retrospective cohort study and a descriptive qualitative study. A total of 180 Hr-TB patient health records were reviewed, and in-depth interviews with 35 participants were conducted. (20 Hr-TB patients and 15 HCWs).
Results: Of the total 180 Hr-TB patients included, we documented unfavourable outcomes in 26.1% of cases, and the *KatG* gene mutation was the most common mutation observed (63.9%). A significant risk of unfavourable outcomes was associated with low adherence and positive sputum at the third-month culture report. In interviewing the stakeholders, major challenges observed were the increased pill burden, delay in diagnosis, shortage of drugs, and lack of staff.
Conclusion: Hr-TB patients have difficulty in adhering to the 6-month levofloxacin regimen, with the need for rigorous early 3-month follow-up and assessment.

1. Introduction

The incidence of Drug-resistant Tuberculosis (DR-TB) has been gradually increasing, with a higher incidence of Isoniazid resistance. The topic of interest, Isoniazid monoresistance tuberculosis (Hr-TB), is in-vitro rifampicin susceptible *Mycobacterium tuberculosis* strains resistant to isoniazid.[1] A drastic increase in Hr-TB has been observed over the last decade, which is solely attributed to the adaptation of algorithms prioritising the detection of Multi-Drug Resistant TB (MDR-TB) by the National Programmes. This is clearly captured in the recent WHO Global TB estimates for Hr-TB vs MDR/RR-TB incidence rates (13.1% vs 3%).[2] A similar difference is observed in India as well (Incidence of Hr-TB vs MD/RR TB: 16% vs 6.19%.[3] Till 2016, in many parts of the world (including India), Hr-TB was treated with standard antitubercular first-line agents. This attempt was proven futile as a higher treatment failure rate and acquired drug resistance were observed.[4] Hence the need for a new drug regime was sensed, and on conditional recommendation, with a meagre strength of evidence, the 6-month all-oral levofloxacin (Lfx) - containing regimen (*Hr-TB regimen recommended by WHO 2017: 6 (H)RZE – Lfx)* was introduced in 2017.[5,6]

In India, in the latter half of 2018, the 6 (H)RZE-Lfx regimen was introduced in a few selected states of India on a programmatic basis, of which Puducherry was one.[7] It has been observed that the cure rate reflected in the India TB reports from 2020 to 2022 remained stagnant at 50–56% over the years, and this proportion is found to be an unsatisfactory response.[8–10] Hence, it was imperative to study this new regimen to determine clinical-socio characteristics, resistance gene, adherence and treatment outcomes and to explore the challenges in the implementation from the perspective of the programme's stakeholders: Isoniazid monoresistant TB (Hr-TB) patients enrolled in the programme and Healthcare workers (HCWs).

* Corresponding author. Department of Pharmacology, JIPMER, Puducherry, India.
E-mail addresses: mirunalini3012@gmail.com, acadcon2019@gmail.com (M. R).
[1] Present affiliation - Chettinad Hospital and Research Institute, Kelambakkam, Chennai.
[2] JIPMER campus, Dhanvanthri nagar, Gorimedu, Puducherry, India.

https://doi.org/10.1016/j.ijtb.2024.05.013
Received 18 January 2024; Received in revised form 29 January 2024; Accepted 31 May 2024
Available online 31 May 2024

2. Methodology

2.1. Study design and setting

This study was designed as a convergent parallel mixed methods study with two parts: a quantitative retrospective study to help investigate the various factors ascertaining the treatment outcome and a qualitative study that looked for challenges influencing the treatment outcome through the perceptions of patients and healthcare workers (See Fig. 1). Under the National Tuberculosis Elimination Programme (NTEP), by Universal Drug Sensitivity Testing (UDST) at the point of suspicion of TB, sputum is tested for the first line TB drugs (HRZE) using Line Probe Assay (LPA). The study was carried out in the outpatient setting of the Puducherry state government hospitals (all PHCs, TB and Chest hospitals and Government chest clinics). Data were collected from two primary sources: 1. Electronic Health Records and 2. Patient case records. The interviews for the qualitative part of the study were conducted in the PHCs that were easily accessible to the participants.

2.2. Study participants and sampling

Approval for the study was acquired after presenting the protocol to the Postgraduate Research Monitoring Committee (PGRMC), the Institute Ethics Committee (IEC), and State TB Operational Research Committee (ORC). This study was conducted from March 2021 to December 2022. All Hr-TB patients from the Union territory of Puducherry enrolled under the NTEP and have been assigned an outcome status after treatment with the 6-month LRZE regimen were included. For the in-depth interview, isoniazid monoresistant tuberculosis patients and healthcare staff working in the NTEP (Medical officers, senior technical supervisors (STS) and TB health visitors (TBHV)) were included. Depending on the design, appropriate sampling methods were employed (Quantitative: total enumerative sampling, qualitative: purposive sampling). A written informed consent was acquired, and the data were deidentified by providing a unique code to each participant.

2.3. Data collection and definitions

To extract the data from the EHR and the patient case records, a standardized questionnaire was implemented. For the in-depth interview, an interview guide was prepared incorporating semi-structured and open-ended questions and was pilot-tested. The outcomes, previously treated status and adherence categories for Hr-TB variables were defined as per the WHO definitions.[1,11]

2.4. Statistical analysis

2.4.1. Quantitative retrospective part

Clinico-social variables, such as participant demographics (e.g., age, gender), treatment characteristics (e.g., delay in initiation of therapy, number of missed doses), and the outcome variable, i.e., the final treatment outcomes (favourable (cured and treatment complete) and unfavourable (treatment failure, lost-to-follow-up and death)) were summarised as mean (standard deviation) and proportions respectively. Following this, to identify the risk factors with the unfavourable outcome, multivariable analysis (log-binomial regression) was done and adjusted relative risk with 95% confidence intervals were reported and a p-value of <0.05 was considered significant. The clinical-social variables were included for bivariate analysis if the p-value for the associated risk with the outcome was <0.2. Statistical Package for Social Sciences (SPSS) software version 29.0 was used for the analysis.

2.4.2. Qualitative content analysis

The in-depth interviews which were audio-recorded were subsequently transcribed verbatim and later translated to English, and then back-translated to the language of the record to confirm the correctness of the translation. A content analysis was carried out using both deductive and inductive approaches. Quotations with important information were marked, and codes were allotted. Similar code groups were identified and grouped into categories. The extracted data were analysed, and a conceptual framework was deduced. The qualitative data

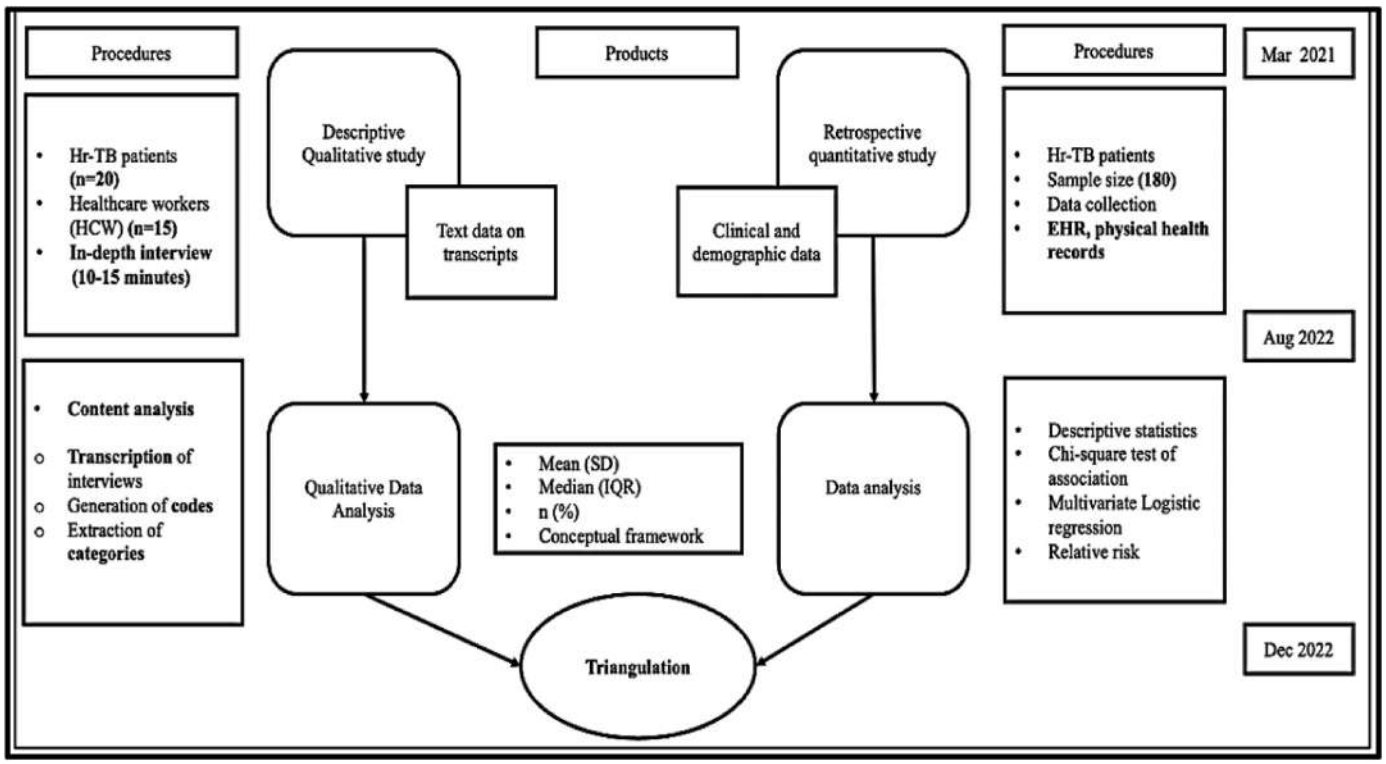


Fig. 1. Mixed-methods framework of the study.

was analysed using ATLAS ti. version 22.0.

3. Results

3.1. Hr-TB patient characteristics

Overall, 180 Hr-TB patients were enrolled in the NTEP from the year 2018–2022. The mean age (SD) of the participants was 44.3 (15.4) years and a mean body mass index (SD) of 18.97 kg/m^2 (4.56). We observed a predominance of males (70.6%) among the HR-TB patients and most of the patients suffered from pulmonary tuberculosis (98.9%). Around 48.9% of the patients were treated with the standard drug-sensitive tuberculosis (DS-TB) regimen for a mean duration (SD) of 37.3 days (35.6). We observed a delay of more than 15 days in initiating HR-TB treatment in one-third of the patients (33.9%). The Hr-TB patients on average consumed twice the number of pills as compared to DS-TB patients, and when assessing adherence in this regard, 50.6% of Hr-TB patients show poor adherence to the regimen of which 41.8% had unfavourable outcomes. The two common genes implicated in Hr-TB are the *KatG* gene and the *InhA* gene and in our study, we observed a higher proportion of KatG gene mutation, contributing to 63.9% of the mutations (Table 1).

3.2. Treatment outcome

Of total, 133 (73.9%) had successful treatment outcomes (and 47 (26.1%) showed an unfavourable outcome (Table 2).

3.3. Associated risk factors

In multivariable analysis, on adjusting for age, gender, BMI, co-morbidities, addiction, and other clinical factors, two factors were independently associated with unfavourable outcomes: Low adherence (aRR (95%CI): 8.82 (3.5–22.2)) and positive sputum culture at 3-month follow-up (aRR (95%CI): 4.30 (2.50–7.37)) (Table 3).

Table 1
Socio-clinical characteristics of INH monoresistant patients on Hr-TB regimen.

Baseline Characteristics	N = 180, n (%)	Clinical Characteristics	N = 180, n (%)
Age category		Previously treated	88 (48.9)
14–30 years	41 (22.8)	Duration of DS-TB regimen (days)[1,2]	37.3 (35.6)
31–45 years	54 (30)	Time-point of Hr-TB treatment initiation (days) ≥ 15 days	61 (33.9)
46–60 years	62 (34.4)	Missed doses (count)[1]	26.9 (34.5)
>60 years	23 (12.8)	Low adherence	91 (50.6)
BMI category		Favourable outcome	35
Underweight (<18.5)	87 (48.3)	Unfavourable outcome	
Normal (18.5–22.9)	77 (42.8)	Number of pills	
Overweight & Obese (>23)	16 (8.9)	Hr-TB regimen	8.18 (1.2)
Education qualification: Primary	126 (70%)	DS-TB regimen as FDC	3.5 (0.8)
Occupation: Daily wage	112 (62.2)	Gene mutation	
BPL	161 (89.4)	Inh A mutation	63 (35)
Married	142 (78.9)	KatG mutation	115 (63.9)
TB supporter	83 (46.1)	Both InhA and KatG	2 (1.1)
Diabetes mellitus	65 (36.1)	Follow-up culture at 3 months[2]	
HIV (negative)	180 (100)	Negative	134 (79.3)
Tobacco/smoking	34 (18.9)	Positive	35 (20.7)
Alcohol intake	36 (20)	Previously treated	88 (48.9)

[1]Mean (SD), BPL- Below Poverty line, Education category (others)- middle, high, higher secondary schooling, and college graduation, BMI categories as per WHO classification of BMI (South Asian population), TB supporter (TB HCW or volunteer, not a family member)), [2]N = 169, *Adherence >90 % of medication, FDC- Fixed Dose Combination.

Table 2
Treatment outcomes of isoniazid monoresistant Tb patients in Pondicherry Union Territory.

Outcomes N = 180	n (%)
Favourable (Treatment success)[a]	133 (73.9)
Cured	128 (71.1)
Treatment complete	5 (2.8)
Unfavourable	47 (26.1)
Treatment failure	14 (7.8)
Lost to follow-up	27 (15)
Death	6 (3.3)

[a] Treatment success implies cured, and treatment completed patients.

3.4. Challenges perceived by stakeholders

Interviews were conducted till data saturation was reached; hence a total of 20 participants from the isoniazid monoresistance tuberculosis (Hr-TB) patients group and 15 participants from the healthcare workers (HCW) group were interviewed.

From the interviews, the three main categories emerged under the theme of challenges in coalescing the codes, viz.: 1. Patient-centred challenges, 2. Regimen-based challenges, and 3. Programme-based challenges (See Fig. 2).

3.5. Patient-centred challenges

Under this category, lack of compliance was a major challenge identified. From the perspective of healthcare workers, poor compliance stemmed from two patient-associated factors viz, alcohol addiction and lack of understanding of the seriousness of the disease. Patients brought in a different perspective on the same challenges. The increased pill burden was found to be a major contributing factor to poor compliance among patients. Patients felt that alcohol debilitated health and hence made it difficult to adapt to the medications.

> "Habits like smoking and alcoholism make it difficult to cope with the strength of the tablets; forgoing it helped me." – 58-year-old male alcoholic

3.6. Regimen-based challenges

Comparing the (6) LRZE regimen with the standard DS-TB regimen, the healthcare workers observed an increased dose of drugs and associated side effects.

> "The drugs in this regimen are given at a higher dose compared to drug-sensitive TB, but the available loose tablets are in small dosages. This can be changed." – Medical officer, Chest Clinic

Patients feel difficulty in transitioning from the DS-TB FDC regimen to the (6) LRZE regimen. Two main problems perceived are the suddenly increased pill burden and the compulsion to take all the tablets in one sitting every day.

> "I was given around 11 tablets to take and advised to take all of them within a span of 10 minutes. I found this to be difficult was very difficult to take the medicines. I was splitting the medications or eating the medicines on alternate days." –58 years old female.

3.7. Programme-based challenges

The Hr-TB regimen is part of a larger programme, the NTEP. This

Table 3
Predictors of unfavourable tuberculosis treatment outcome in Isoniazid monoresistant tuberculosis patients, Puducherry, India.

Determinants	Hr-TB case records N = 180 n (%)	Unfavourable outcomes n (%)	Unadjusted Relative Risk (95% CI)	Adjusted Relative Risk (95% CI)	p value
Age category (years)					
14-30	41	5 (10.6)	1	1	
31-45	54	23 (48.9)	3.49 (1.45–8.40)	1.65 (0.65–4.20)	0.296
46-60	62	15 (31.9)	1.98 (0.78–5.04)	1.30 (0.46–3.67)	0.615
>60	23	4 (8.5)	1.42 (0.43–4.79)	0.56 (0.12–2.70)	
Gender					
Male	126	40 (85)	2.40 (1.15–5.02)	1.14 (0.53–2.44)	0.74
Female	53	7 (15)	1	1	
BMI category (kg/m^2)					
Underweight (<18.5)	87	28 (59.6)	1.32 (0.44–3.92)	1.60 (0.58–4.43)	0.37
Normal (18.5–22.9)	77	14 (29.8)	1.53 (0.52–4.50)	2.06 (0.80–5.40)	0.14
Overweight & Obese (≥23)	16	5 (10.6)	1		
Education					
Primary	126	39 (83)	2.09 (1.05–4.17)	2.05 (0.47–8.82)	0.34
Others	54	8 (17)	1	1	
Occupation					
Unskilled	112 (62.2)	35 (74.5)	2.56 (1.08–6.09)	1.12 (0.24–5.22)	0.89
Salaried	27 (15)	7 (14.9)	2.13 (0.75–6.01)	2.50 (0.70–8.70)	0.16
Unemployed	41 (22.8)	5 (10.6)	1	1	
Socioeconomic status					
APL	19 (10.6)	3 (6.4)	1		
BPL	161 (89.4)	44 (93.6)	1.73 (0.59–5.04)		
Marital status					
Married	142 (78.9)	83	1.30 (0.67–2.55)		
Single	38 (21)	17			
TB supporter					
Present	83 (46.1)	39 (83)	1		
Absent	97 (53.9)	8 (17)	1.26 (0.76–2.09)		
Diabetes mellites					
Absent	115 (63.9)	34 (72.3)	1.48 (0.84–2.60)	1.52 (0.82–2.82)	0.184
Present	65 (36.1)	13 (27.7)	1		
Tobacco/smoking					
Absent	146 (81.1)	34 (72.3)	1	1	
Present	34 (18.9)	13 (27.7)	1.64 (0.98–2.76)	1.41 (0.79–2.52)	(0.25)
Alcoholism					
Absent	36	33 (70.2)	1	1	
Present	144	14 (29.3)	1.70 (1.02–2.81)	1.20 (0.66–2.14)	0.56
Site of disease					
Pulmonary	178 (98.9)	46 (97.9)	1		
Extra-pulmonary	2 (1.11)	1 (2.1)	1.93 (0.5–7.91)		
Status of patient at diagnosis of HrTB					
New	92 (51.1)	24 (26)	1		
Previously treated	88 (48.9)	23 (26)	1.002 (0.61–1.64)		
Time-point of treatment initiation (days)					
< 7 days	65	13 (27.7)	1		
7–14 days	53	21 (44.7)	1.09 (0.62–1.92)		
≥ 15 days	61	13 (27.7)	0.77 (0.41–1.43)		
Adherence					
Low adherence	73	35 (74.5)	7.3 (3.42–15.5)	8.82 (3.5–22.2)	<0.001*
Adherence	107	12 (25.5)	1	1	
Gene mutation					
KatG	115	26 (55.3)	1	1	
InhA	63	20 (42.6)	1.404 (0.86–2.30)	1.20 (0.73–1.94)	0.73
Both	2	1 (2.1)	2.21 (0.53–9.21)	1.42 (0.30–7.3)	0.42
Outcome at 3 months					
Negative	134 (79.3)	16 (43.2)	1	1	
Positive	35 (20.7)	21 (56.8)	5.025 (2.95–8.57)	4.30 (2.50–7.37)	<0.001*

APL- Above Poverty Line, BPL Below Poverty Line, HrTB- Isoniazid monoresistant tuberculosis; Unfavourable outcome- Treatment failure, lost to follow-up, and death; CI- Confidence Interval, *p-value <0.05, BMI- Body Mass Index, Kg/m^2, Treatment supporter – TB HCW/Volunteer/Peer (Not a family member).

change over to a 6-month levofloxacin regimen has shown an evident improvement in the cure rate. However, the major drawbacks observed were in the programme implementation viz, delay in diagnosis and unavailability of large denomination of drugs. HCWs and patients perceived an increased turnaround time with regard to the Hr-TB diagnostic test.

"*The LPA report comes within 10 to 15 days, and we start therapy at least by one month after initial diagnosis*."-TB health visitor.

"After diagnosing TB, two additional tests were done. I was started on four similar-looking red colour tablets and I took them for 3 weeks. The second test result took about a month to come." – 58 years old diabetic.

A major negative aspect of the programme is the shortage of drugs, especially higher denomination of drugs, e.g., levofloxacin 1000 mg, this leads to increased pill burden as lower denomination, e.g., levofloxacin 250 mg, are dispensed for higher doses.

Other challenges voiced are the lack of adequate staff which is reflected in a lack of stringent follow-up and counselling of patients.

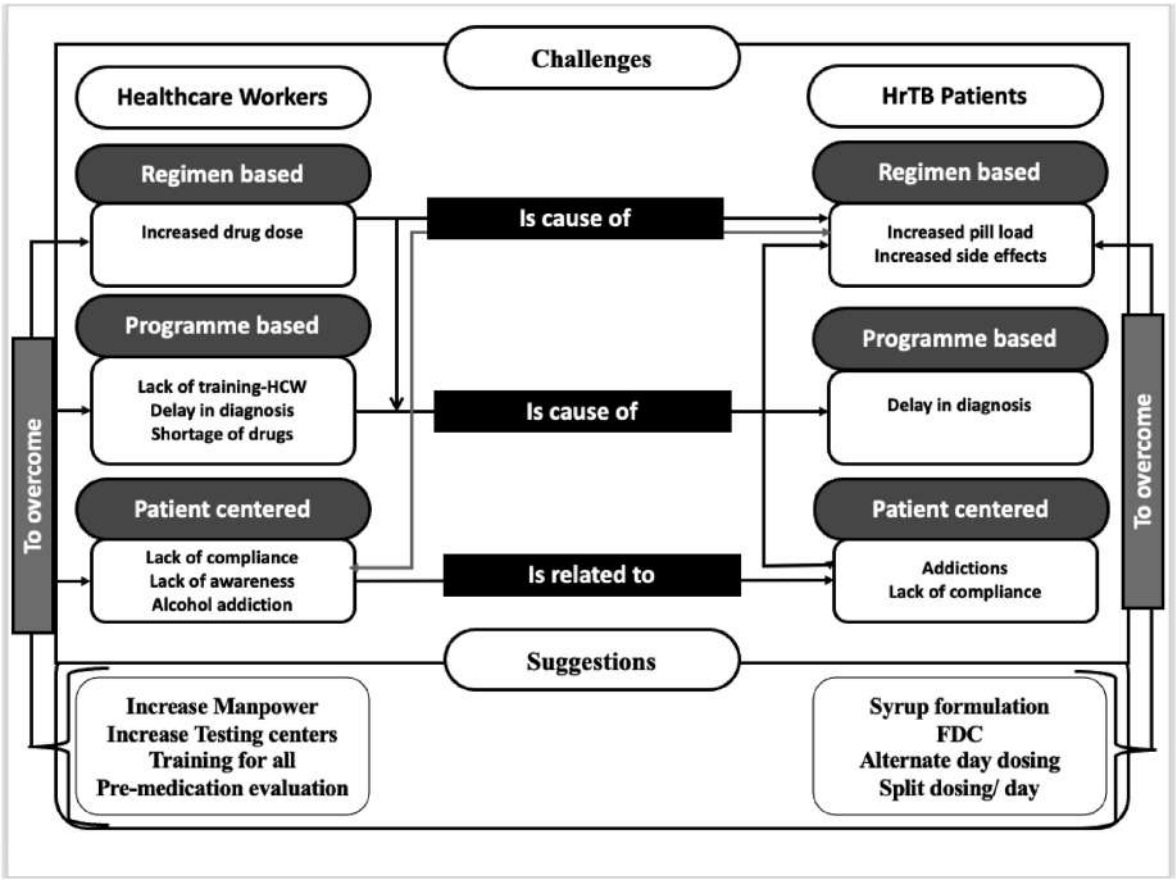


Fig. 2. Conceptual framework of the perceptions of isoniazid mono-resistant tuberculosis patients and healthcare workers on challenges faced with the all-oral levofloxacin regimen.

"In my PHC we have 60 patients whom I cater to. I feel manpower is an important factor."- TB health visitor]

4. Discussion

Our study is conducted in the Union Territory of Puducherry, India one of the very few states where the (6) LRZE regimen was first introduced on a programmatic basis. In our study, we documented a cure rate of 71%, a treatment success rate of 74%, and an unfavourable outcome of 26 % with the new 6-month LRZE regimen for Hr-TB, based on the data collected from the 180 patient health records from the year 2018–2022. The gene predominantly found mutated in this population was the *KatG* mutation (115, 63.9%). A delay of more than 15 days was documented in 35.6% of the patients and poor adherence was observed in 50.6% of the patients. In the qualitative part, the challenges recognised by the healthcare workers and patients could be categorised as regimen-based, programme-based, and patient-centred.

Our study showed a preponderance in outcomes similar to the National Consensus and a few other studies.[10–12] However, on comparing the cure rate, we observed a higher cure rate, which could be due to the increased lost-to-follow-up seen in other studies.[10,12] For instance, a retrospective study by Bachir et al. observed a cure rate of 10% and a treatment success rate of 76%.[12] This discrepancy is observed in our national census as well. This could be due to the grey area of treatment completed status, wherein a patient has successfully completed the regimen without proof of cure. This creates lacunae in the knowledge of outcome status.

The two resistant genes most commonly found to confer isoniazid resistance are *KatG* and *InhA* genes, of which the former is associated with higher rates of unfavourable outcomes. A predominance of *KatG* gene mutation was observed, similar to other studies.[13,14] However, this pattern is not reflected in studies from other geographic distributions, confirming its predominance in high disease-burden regions.[15]

Looking into the risk factors for unfavourable outcomes, we documented a higher proportion of malnourished, diabetic, smokers and alcoholics as in other studies.[16,17] A higher proportion of Hr-TB patients with previous exposure to ATT, a known risk factor, has been reported.[16] However, while we observed an equal proportion, in our study, those who failed the standard TB regimen constituted 47.8% of the previously treated patients[18] and were found to have a 16% higher proportion of unfavourable outcomes than patients who were given an extended period of standard ATT coverage (provided during the time gap till Hr-TB treatment initiation).

In our study, a significant risk of unfavourable outcomes was associated with positive culture at the 3rd-month follow-up (RR [95%CI]: 4.3 [2.5–7.37]). From the PMDT guidelines, one can appreciate that a third-month sputum culture is the first definite point of assessing treatment progress. This time point is crucial, as based on this report, reassessment for MDR TB is done. Low adherence was observed to be a significant risk for unfavourable outcomes among the patients (RR [95% CI]: 8.82 [3.5–22.2]) in our study and was similarly reported by Bachir et al.[16] As per the WHO DOTS programme, adherence to >90% is desired for favourable treatment outcomes. In our study, of the patient with poor adherence, only 41.8% had an unfavourable outcome. A single-point cut-off to determine adherence in TB programmes which is based on self-reporting predominantly is not robust,[19] as in our study around 20% of the favourable outcome with poor adherence lie between the 80–90% cut-off.

This study was designed as a mixed-methods study to determine the imperative factors perceived as challenges for implementing the new Hr-

TB regimen. From our observation, we could appreciate that the challenges faced by the HCWs and the patients are not stand-alone but are interlinked. The low adherence observed as a significant risk in the study could be attributed to the difficulties the regimen imposed, especially the higher pill burden with once-a-day dosing. We observed that from the programme perspective, the major contributing factor towards the increased pill burden was a shortage of higher denominations of drugs. Furthermore, patient characteristics like alcohol addiction, which was perceived as the major cause driving poor care and increased dropout, was also linked with poor treatment adherence. From the qualitative perspective, the stakeholders have observed that most of the patients achieve symptom relief in the 3rd month, and adherence to the regimen is found to drop. Hence this time point should be rigorously monitored, and adequate support and counselling should be provided to improve adherence.

We have tried to assess the 6-month all-oral LRZE regimen from both a quantitative point of view and a qualitative point of view, which could be considered a strength of this study. The mixed-methods study design is an upcoming model employed to assess programme implementation in health science. Being a record-based study, data loss was inevitable; however, we attempted to overcome this by keeping a total enumerative sampling. A qualitative component provided insight into various challenges the stakeholders faced while implementing this recently introduced regimen, and this knowledge could be extrapolated to model studies that could provide insight into future research and programme recommendations.

Funding

JIPMER intramural fund, JIPMER, Puducherry, India.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. World Health Organization. *Definitions and Reporting Framework for Tuberculosis – 2013 Revision: Updated December 2014 and January 2020* [Internet]. Geneva: World Health Organization; 2013 [cited 2023 Jan 15]. Available from: https://apps.who.int/iris/handle/10665/79199.
2. World Health Organization. *Global Tuberculosis Report 2020 [Internet]*. Geneva: World Health Organization; 2020 [cited 2023 Jan 15]. Available from: https://apps.who.int/iris/handle/10665/360605.
3. [Internet]. *Guidelines for Programmatic Management of Drug-Resistant Tuberculosis in India*. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2021 [cited 2023 Jan 15] Available from: https://tbcindia.gov.in/showfile.php?lid=3590.
4. Gegia M, Winters N, Benedetti A, van Soolingen D, Menzies D. Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. *Lancet Infect Dis*. 2017;17:223–234.
5. World Health Organization. *WHO Treatment Guidelines for Isoniazid-Resistant Tuberculosis: Supplement to the WHO Treatment Guidelines for Drug-Resistant Tuberculosis* [Internet]. Geneva: World Health Organization; 2018 [cited 2023 Jan 13]. Available from: https://apps.who.int/iris/handle/10665/260494.
6. [Internet]. *Guidelines for Programmatic Management of Drug Resistant Tuberculosis in India*. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2017 [cited 2023 Jan 15] Available from: https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4780&lid=3306.
7. Deepa D, Achanta S, Jaju J, et al. The impact of isoniazid resistance on the treatment outcomes of smear positive Re-treatment tuberculosis patients in the state of Andhra Pradesh, India. *PLoS One*. 2013;8, e76189.
8. India TB Report. *National Tuberculosis Elimination Programme. Annual Report* [Internet]. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2020, 2020. [cited 2023 Jan 15] Available from: https://tbcindia.gov.in/showfile.php?lid=3538.
9. India TB report. *National Tuberculosis Elimination Programme. Annual Report* [Internet]. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2021, 2021. [cited 2023 Jan 15] Available from: https://tbcindia.gov.in/showfile.php?lid=3587.
10. India TB Report. *National Tuberculosis Elimination Programme. Annual Report* [Internet]. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2022, 2022. [cited 2023 Jan 15] Available from: https://tbcindia.gov.in/index1.php?lang=1&level=1&sublinkid=5613&lid=3658.
11. World Health Organization. *Guidelines for Treatment of Drug-Susceptible Tuberculosis and Patient Care [Internet]. 2017 Update* [cited 2022 Dec 13]. Geneva: World Health Organization; 2017:80. Available from: https://apps.who.int/iris/handle/10665/255052.
12. Sayfutdinov Z, Kumar A, Nabirova D, et al. Treatment outcomes of isoniazid-resistant (rifampicin susceptible) tuberculosis patients in Uzbekistan, 2017-2018. *Int J Environ Res Publ Health*. 2021;18:2965.
13. Bachir M, Guglielmetti L, Tunesi S, et al. Isoniazid-monoresistant tuberculosis in France: risk factors, treatment outcomes and adverse events. *Int J Infect Dis*. 2021;107:86–91.
14. Binkhamis KM, Bahatheg MA, Altahan FA, et al. Prevalence and outcome of isoniazid-monoresistant tuberculosis at a university hospital in Saudi Arabia. *SMJ*. 2021;42:636–642.
15. Wilson M, O'Connor B, Matigian N, Eather G. Management of isoniazid-monoresistant tuberculosis (Hr-TB) in Queensland, Australia: a retrospective case series. *Respir Med*. 2020;173, 106163.
16. Charan AS, Gupta N, Dixit R, et al. Pattern of InhA and KatG mutations in isoniazid monoresistant Mycobacterium tuberculosis isolates. *Lung India*. 2020;37:227.
17. Alagappan C, Sunil Shivekar S, Brammacharry U, et al. Prevalence of mutations in genes associated with isoniazid resistance in Mycobacterium tuberculosis isolates from re-treated smear-positive pulmonary tuberculosis patients: a meta-analysis. *J Glob Antimicrob Resist*. 2018;14:253–259.
18. Bollela VR, Namburete EI, Feliciano CS, Macheque D, Harrison LH, Caminero JA. Detection of katG and inhA mutations to guide isoniazid and ethionamide use for drug-resistant tuberculosis. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis*. 2016;20:1099–1104.
19. Forman LS, Lodi S, Fatch R, et al. Agreement between measures of adherence to isoniazid preventive therapy among people with HIV in Uganda. *Open Forum Infect Dis*. 2022;9, ofac516.

Indian Journal of Tuberculosis 71 (2024) S97–S100

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

The Longer the Therapy, the Worse the Severity of the Adverse Drug Reactions that Occur in Drug-Resistant Pulmonary Tuberculosis Patients

Rinto Susilo [a,*], Dwini Nafertari Azzahra [b], Iin Indawati [a], Arsyad Bachtiar [a], Indah Setyaningsih [a], Didi Rohadi [a], Tomi [a], Kustini [b]

[a] Sekolah Tinggi Farmasi Muhammadiyah Cirebon, Jl. Cideng Indah Nomor 3, Cirebon, Jawa Barat, Indonesia
[b] Department of Pharmacy, Gunung Jati Hospital, Jl. Kesambi No. 56, Cirebon, Jawa Barat, Indonesia

ARTICLE INFO

Keywords:
Drug resistant pulmonary TB (DR-TB)
Length of therapy
Regimen of therapy
Severity of adverse drug reactions (ADRs)

ABSTRACT

Background: It is estimated that drug-resistant (DR) Tuberculosis (TB) (DR-TB) patients in Indonesia are 2.40% of all new TB patients and 13% of previously treated TB patients with a total incidence of DR-TB cases of 24,000 people. The adverse drug reactions (ADRs) of DR-TB are still a problem that can certainly affect the success of therapy. The aim of this study was to determine the correlation between the length of therapy and regimen therapy of DR-TB with the severity of ADRs.
Methods: Data collection was carried out retrospectively on the medical records of DR-TB patients in 2020–2021 and sampling used a purposive sampling technique that complied with the inclusion criteria.
Results: Of the 86 patients, the majority of DR-TB patients in X Hospital were 26–45 years old 35 (40.7%), 52 (60.5%) male, the most common comorbid was type II DM, 19 (22.1%), and the most nutritional status was malnutrition as much as 39 (45.3%). The most common type of ADR was hyperuricemia in 31 (36.0%). The results of the correlation analysis showed that there was a relationship between the length of therapy and the severity of ADRs ($\rho = 0.002$) and there was no relationship between the type of therapy regimen and the severity of ADRs ($\rho = 0.184$).
Conclusion: The longer DR-TB therapy, the higher the severity of ADRs and there is no relationship between the type of therapy regimen and the severity of ADRs.

1. Introduction

Tuberculosis (TB) is an infectious disease that is the main cause of health problems and is also one of the 10 biggest causes of death in the world, as well as the main cause of death due to a single infectious agent (ranked above HIV/AIDS).[1] M.Tuberculosis will be in the air by means of being transmitted through saliva splashes from people suspected of having DR-TB when coughing, sneezing, talking, or singing. The water splash will be inhaled, then enter the lungs and spread to the alveoli and M. Tuberculosis will multiply. M.Tuberculosis can also enter the blood and then spread to other parts of the body (brain, lungs, bones, spine, kidneys, larynx) which allows for the proliferation of M. Tuberculosis. Drug-Resistant Tuberculosis is when a patient suspected of having TB develops resistance to isoniazid and rifampicin, with or without other first-line anti-TB drugs such as isoniazid, rifampicin, ethambutol, and streptomycin 2. In Indonesia, it is estimated that DR-TB patients account for 2.40% of all new TB patients and 13% of previously treated TB patients with a total incidence of DR-TB cases of 24,000 people.[2] The therapy regimen for second-line TB patients can be adjusted by a team of DR-TB experts if there is a change in the patient's laboratory test results. Following WHO stipulations in 2019, DR-TB treatment no longer uses kanamycin or capreomycin injection drugs and is replaced with bedaquiline. Treatment is divided into two, namely a short-term therapy regimen for 9–11 months and a long-term for 18–24 months. The short-term DR-TB therapy regimen consists of 7 types of drugs at the initial stage and 4 types of drugs at the advanced stage. Long-term DR-TB therapy is given to patients who cannot get short-term therapy. Long-term DR-TB therapy combined treatment can be modified according to the patient's condition which is shown to increase the effectiveness and safety of treating DR-TB patients.[2]

2. Materials and Methods

The research will be conducted using non-experimental

* Corresponding author.
E-mail address: rintosusilo88@gmail.com (R. Susilo).

https://doi.org/10.1016/j.ijtb.2024.06.001
Received 1 February 2024; Received in revised form 30 May 2024; Accepted 4 June 2024
Available online 10 June 2024

Table 1
Age of patients with DR-TB disease.

Age (years)	Frequence	Percentage (%)
15–25	29	33,7
26–45	35	40,7
>46	22	25,6
Total	86	100

(observational) methods. Data collection for DR-TB patients for 2020–2021 was carried out in February 2022. The study was conducted from December 2021 to May 2022 at the Integrated Pulmonary Outpatient, X Hospital, Cirebon City. The population used in this study is all medical record data for DR-TB patients in 2020–2021 who have started, are currently or have finished treatment or are currently undergoing DR-TB treatment. The sample used is medical record data of DR-TB patients for 2020–2021 who meet the inclusion criteria. The sampling technique used purposive sampling technique. The inclusion criteria in this study were: a) patients aged 15–79 years, b) patients who started, were, or have started treatment from January 2020 to December 2021, c) patients who experienced complaints during treatment. If data on ADRs that patients complain about have been obtained, these data can then be analyzed for the severity of ADRs using the Hartwig & Siegel, 1992 scale. After obtaining these data, a correlation test was carried out using Spearman statistical analysis.

The data obtained was analyzed using SPSS version 25. Univariate analysis was carried out to get an overview of the patient's characteristics presented in the form of frequency and percentage tables.[3] In this study, univariate analysis was carried out to determine the characteristics of DR-TB patients including age, sex, comorbidities, and nutritional status. The duration of therapy is seen from the time of the appearance of ADRs which is marked by the month to which the patient experiences complaints about the drugs he is taking. The combined type of short-term therapy is carried out with a duration of treatment of 9–11 months, while the combination of long-term therapy is carried out with a duration of treatment of 18–24 months.[2] The type of combined therapy is determined by the doctor as written in the medical record, then the ADRs that appear are then analyzed by the Naranjo algorithm. If data on ADRs that patients complain about have been obtained, these data can then be analyzed for the severity of ADRs using the Hartwig & Siegel, 1992 scale. After obtaining these data, a correlation test was carried out using Spearman statistical analysis.

3. Results and Discussion

3.1. Patient Characteristics

In this study, sampling was carried out using the purposive sampling method in patients with DR-TB disease at X Hospital in Cirebon in 2020–2021. Medical record data that matched the inclusion criteria in this study were 86 medical record data. The analysis used was univariate analysis including age, sex, comorbidities, and nutritional status.

In **Table 1**. it is stated that the majority of the age range of DR-TB patients at X Hospital were aged 26–45 years with a total of 35 (40.7%) patients, aged 15–25 years as many as 29 (33.7%) patients, and >46 years there are 22 (25.6%). This study is similar to that of Pratiwi et al. (2016)[4] and Lu et al. (2019)[5] which stated that the age range of DR-TB patients is below 40 years. This can be related to the high activity outside the home compared to activities inside the home so that it is vulnerable to disease transmission and transmitting the disease to other people.

Patients with DR-TB disease based on gender in this study found data that male sex suffered the most DR-TB disease, namely 52 (60.5%) patients and the rest were female sex, there were 34 (39.5%) patients. This is similar to previous research which stated that the majority of male sex suffers from DR-TB disease.[4] WHO states that in 2019, around 10 million people in the world suffer from DR-TB disease where as many as 56% are dominated by men, 32% women and 12% children.[1] This incident is often associated with men leaving the house more often than women to earn a living, heavy workloads, lack of rest, or unhealthy lifestyles, such as smoking and drinking alcohol.[6](Table 2).

Table 2
Sex of patients with DR-TB disease.

Sex	Frequence	Percentage (%)
Man	52	60,5
Woman	34	39,5
Total	86	100

Table 3
Comorbid patients with DR-TB disease.

Comorbid	Frequence	Percentage (%)
HIV + DM II	1	1,2
DM II	19	22,1
No Comorbid	66	76,7
Total	86	100

HIV Human Imunodeficiency Virus; DM II: Diabetes Mellitus Type 2.

Table 4
Nutritional status of patients with DR-TB disease.

Nutritional Status	Frequence	Percentage (%)
Malnutrition	39	45,3
Normal	36	41,9
Obesity	11	12,8
Total	86	100

Diabetes Mellitus is the biggest comorbid in this study. **Table 3.** states that the characteristics of patients with DR-TB disease with the most comorbidities were type II DM, namely 19 (22.1%) patients and 1 (1.2%) patient suffering from HIV and DM, but the majority of the entire sample were patients who did not have comorbidities of 66 (76.7%). In line with research conducted in Bangladesh which revealed that the most comorbid were type II DM but more had no comorbidities.[7] Treatment of DR-TB with comorbid type II DM becomes difficult by controlling blood sugar levels.[8] HIV comorbidities in a study conducted in Medan revealed the possibility of HIV occurrence in DR-TB patients due to the patient's bad lifestyle such as drug use and unsafe sexual relations.[9] Patients with diabetes mellitus have impaired immunity and can increase the side effects of DR-TB drugs.[10]

Data has been obtained that patients with DR-TB disease based on nutritional status are mostly malnourished with a total of 39 (45.3%) patients. Patients with normal nutritional status were 36 (41.9%), and patients who were obese were 11 (12.8%). Poor nutritional status has a 6 times greater risk of experiencing DR-TB disease.[6] If a person is malnourished, the body's resistance decreases and he continues to breathe, which contains TB germs, he will be more easily infected and

Table 5
Types of adverse drug reactions appears based Naranjo algorithm score.

Types of ADRs	*Probable*	*Possible*	Total
Cardiovascular Syndrome	1[1,2]	0	1(1,2%)
Neurophaty Perifer	1(1,2%)	2(2,3%)	3(3,5%)
Hearing Syndrome	11(12,8%)	0	11(12,8%)
Sleep Disorders	0	1(1,2%)	1(1,2%)
Gastrointestinal Disorders	27(31,4%)	0	27(31,4%)
Liver Function Abnormalities	3(3,5%)	0	3(3,5%)
Hyperuricemia	31(36,0%)	3(3,5%)	34(39,5%)
Optic Neuritis	1(1,2%)	0	1(1,2%)
Arthralgia	1(1,2%)	0	1(1,2%)
Electrolyte Disorders	3(3,5%)	1(1,2%)	4(4,7%)
Total	**79**	**7**	**86**

can cause the TB germs that were previously asleep to become active. WHO states that many new cases of TB are caused by malnutrition.[1](Table 4).

4. Types of Adverse Drug Reactions

Complaints contained in the patient's medical record were analyzed using the Naranjo algorithm score to determine whether these complaints were caused by ADRs.

The types of ADRs that appear based on the Naranjo algorithm score contained in Table 5 show that hyperuricemia is the most common ADRs, namely 34 (39.5%). According to research conducted at Dr. SoetomoHospital, o Indonesia stated that hyperuricemia was the most commonADRs suffered by DR-TB patients, followed by digestive disorders and ototoxicity.[11] It is known that drugs that have ADRs of hyperuricemia are pyrazinamide and ethambutol.[11] Pyrazinamide is a uric acid retention agent that causes >80% reduction in renal clearance at a dose of 300 mg daily, whereas ethambutol increases uric acid by reducing uric acid clearance.[12] In the treatment of DR-TB, the dose of pyrazinamide used is 400 mg and 500 mg.[2] The antagonist drug used to treat hyperuricemia is allopurinol.[13] Followed by the types of ADRs experienced by patients, namely gastrointestinal disturbances in 27 (31.4%) patients such as nausea and vomiting, hearing loss such as ringing in the ears or reduced hearing by 11 (12.8%), electrolyte disturbances such as hypokalemia there were 4 (4.7%) patients, liver function abnormalities such as high SGOT and SGPT values from normal values in 3 (3.5%) patients, heart problems experienced in 1 (1.2%) patients, there were 3 (3.5%) patients experienced peripheral neuropathy, optic neuritis in 1 (1.2%), arthralgia in this study there was 1 (1.2%) patient, and sleep disturbance occurred in 1 (1.2%) patient.

5. Severity of Adverse Drug Reactions

The severity of ADRs was assessed using the Hartwig scale which has been translated and validated in Indonesian.[14] In this study, the severity of ADRs was mostly at level 3 in 51 (59.3%) patients. Level 1 was 33 (38.4%) and grade 4 was 2 (2.3%) patients. ADRs suffered by the majority of patients can be resolved with the administration of symptomatic drugs. In previous studies, the severity was grouped as grades 1 and 2 were grouped at less severe severity, grades 3 and 4 at moderate severity, and grades 5, 6, and 7 at severe severity.[15] In this study, an analysis of the severity level was carried out using a definition that was in accordance with the original but in Indonesian.[14]

6. Correlation of Length of Therapy with the Severity of Adverse Drug Reactions

The most frequent occurrence of ADRs occurred after 1 month from the start of treatment in 23 (26.7%) patients. At 2nd month there were 17 (19.8%), at 3rd month there were 8 (9.3%) patients. The 6th month there were 7 (8.1%) patients. There were ADRs in 6 (7.0%) patients at 4th and 6th month (7.0%) patients at 5th month. 4 (4.7%) patients showed ADRs at 7th month, 11th month there were 3 (3.5%) patients, there were 4 (4.7%) patients >12 months and the 12th month there were 2 (2.3%) patients experiencing ADRs. There was 1 (1.2%) patient experienced ADRs at 10 months. In this study there was no data indicating the occurrence of ADRs at 9 months. Research reveals that hearing loss can occur as soon as 2 months and is known no later than 12 months after giving DR-TB treatment.[15] Adverse Drug Reactions of liver dysfunction can occur within 2 months, 1 month and 4 months when DR-TB treatment is started.[16] Arthralgias may occur at 1 month, 4 months, and 6 months while treatment is ongoing.[15]

The results of bivariate analysis were performed using the Spearman test. The decision of this test is if the value of Sig. > 0.01 then Ho is accepted, but if the Sig. < 0.01 then Ho is rejected and H1 is accepted. In the results of the correlation test between the length of therapy and the severity of ADRs, the value of Sig. 0.002 < 0.01 where the rate states that Ho is rejected and there is a relationship between the length of therapy and the severity of ADRs.

Table 6
Severity of ADRs in DR-TB patients.

Severity	Frequence	(%)
Level 1	33	38,4
Level 3	51	59,3
Level 4	2	2,3
Total	**86**	**100**

The duration of therapy in this study was defined as determining the appearance of ADRs for the length of therapy that patients had undergone and categorized in months. The results of this study are different from previous research conducted by Pratiwi et al. (2016)4. This can happen due to differences in methods when collecting data, data processing, and the number of samples in the study.

7. Correlation Types Regimen Therapy with the Severity of Adverse Drug Reactions

In this study, the most widely used combination of therapy was long-term therapy namely Individual Therapy Regimen (ITR) of 54 (62.8%) patients using this guide, and the rest used short-term therapy namely Short Therapy Regimen (STR) of 32 (37.2%). Short Therapy Regimen are carried out for 9–11 months, while Individual Therapy Regimen are carried out for 18–24 months.[2] Individual Therapy Regimen can be modified according to the patient's condition.[2] The composition of the Short Therapy Regimen is standardized, but can be modified in certain conditions such as the occurrence of ADRs, where ethionamide can be replaced with prothionamide or levofloxacin replaced with moxifloxacin 2. For the long-term type of therapy regimen or ITR, the number of patients is greater than for the Short Therapy Regimen (STR), because the ITR can be adjusted to the patient's clinical condition and the criteria for the type of patient are broader, whereas it is different from the STR where the criteria are stricter, such as2.

1) Not resistant to fluoroquinolones
2) No contact with pre/XDR TB patients
3) Never received second-line anti-tuberculosis drugs for ≥1 month
4) There is no resistance or suspected ineffectiveness to anti-tuberculosis drugs in short-term combinations (except INH resistance with inhA or katG mutations).
5) Not currently pregnant or breastfeeding
6) Not a case of severe pulmonary TB
7) Not a case of severe extrapulmonary TB
8) DR TB patients (pulmonary or extrapulmonary) with HIV
9) Children aged more than 6 years

Table 7
Length of therapy with the severity of adverse drug reactions.

Incidence Rate of ADRs	Number of Patients	(%)
<1 Month	5	5,8
1st Month	23	26,7
2nd Month	17	19,8
3rd Month	8	9,3
4th Month	6	7,0
5th Month	6	7,0
6th Month	7	8,1
7th Month	4	4,7
8th Month	1	1,2
9th Month	3	3,5
10th Month	2	2,3
>10 Months	4	4,7
Total	**86**	**100**

Table 8
Correlation of length of therapy with the severity of adverse drug reactions.

Variable	Severity of Adverse Drug Reactions		
	Frequence	Correlation Coefficient	$\rho*$
Length of Therapy	86	0,306*	0,002

*Significance of Correlation with the level of 0.01.

Table 9
Types of the regimen therapy in DR-TB

Types the Regimen Therapy	Frequence	(%)
STR*	32	37,2
ITR**	54	62,8
Total	**86**	**100**

*STRv = Short Therapy Regimen; **ITR = Individual Therapy Regimen.

Table 10
Correlation types the regimen therapy with the severity of ADRs.

Variable	Severity of Adverse Drug Reactions		
	Frequencies	Correlation Coefficient	$\rho*$
Types the Regimen Therapy	86	0,098*	0,184

*Significance of Correlation with the level of 0.01.

In 2019, Short Therapy Regimen was renewed by WHO where the kanamycin injection drug was discontinued due to the occurrence of ADRs, namely hearing loss and was replaced with the drug bedaquiline. Currently, short-term co-administered drugs consist of bedaquiline, fluoroquinolones, clofazimine, ethionamide, high-dose INH, pyrazinamide, and ethambutol.[17]

The correlation test of the type of therapy combination with the severity of ADRs showed a significant value. $0.184 > 0.01$ where the figure states that Ho is accepted so there is no relationship between the type of therapy regimen and the severity of ADRs. This is possible because the severity of ADRs that have occurred in patients does not adjust to the type of treatment regimen the patient is undergoing, meaning that ADRs will occur and there is no difference in the severity of ADRs between patients with the type of long-term therapy regimen with the type of short-term therapy regimen(Tables 6–10).

8. Conclusion

The longer the therapy, the higher the severity of ADRs in DR-TB patients, whereas there is no relationship between the type of therapy regimen and the severity of ADRs. Although there is no relationship between the type of combination therapy and the severity of ADRs, the type of therapy combination shows a different duration of therapy, so that short-term therapy is better in terms of the severity of ADRs.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rinto Susilo reports financial support was provided by School of Pharmacy Muhammadiyah Cirebon. Rinto Susilo reports a relationship with School of Pharmacy Muhammadiyah Cirebon that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Authors thank the X Hospital, Cirebon, for being willing to become the assessment team and provide research facilities and ethical clearance. Also, we appreciate LPPM STF Muhammadiyah Cirebon for the support of funds.

Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijtb.2024.06.001.

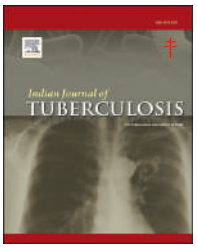
References

1. https://reliefweb.int/report/world/global-tuberculosis-report-2020?gad_source=1&gclid=CjwKCAjwyJqzBhBaEiwAWDRJVFB0w3asLJ83mZIeW15di0YB02ExKxekJRiUtyqpqNwhxPpqzsqt0xoCsdsQAvD_BwE.
2. Kemenkes RI. *Petunjuk Teknis Penatalaksanaan Tuberkulosis Resisten Obat Di Indonesia*. 2020.
3. Dania H, Faridah IN, Rahmah KF, Abdulah R, Barliana MI, Perwitasari DA. Hubungan Pemberian Terapi Antipsikotik terhadap Kejadian Efek Samping Sindrom Ekstrapiramidal pada Pasien Rawat Jalan di Salah Satu Rumah Sakit di Bantul, Yogyakarta. *Indones J Clin Pharm.* 2019 Mar;8(1).
4. Pratiwi N, Yowani S, Sajinadiyasa I. Hubungan Lama penggunaan obat anti tuberkulosis dengan efek samping pada pasien Tb mdr rawat jalan di rsup sanglah denpasar. *Arch Community Heal*. 2016;3(2):39–48.
5. Lu Z, Jiang W, Zhang J, et al. Drug resistance and epidemiology characteristics of multidrug-resistant tuberculosis patients in 17 provinces of China. *PLoS One*. 2019 Nov;14(11).
6. Nunkaidah M, Lestari H, Afa J. Prevalensi risiko kejadian tuberkulosis multi drug resistance (Tb-Mdr) di kabupaten muna tahun 2013 – 2015. *J Ilm Mhs Kesehat Masy Unsyiah*. 2017;2(6), 198308.
7. Siddik AB, Hossain MM, Zaman S, et al. Descriptive epidemiology of multidrug resistance tuberculosis (MDR-TB) in Bangladesh. *J Tubercul Res*. 2018;6(4):292–301.
8. Dwiastuti I, Djano NAR. Studi deskriptif pasien Tb Mdr di sulawesi selatan. *An-Nadaa J Kesehat Masy*. 2020;6(2).
9. Iswa F. *Efek Samping Regimen Individual Pengobatan Tb Resistan Elektrolit Pada Pasien Tb Paru Resistan Obat*. 2019:108.
10. Ketema DB, Muchie KF, Andargie AA. Time to poor treatment outcome and its predictors among drug-resistant tuberculosis patients on second-line anti-Tuberculosis treatment in Amhara region, Ethiopia: retrospective cohort study. *BMC Publ Health*. 2019;19(1):1–10.
11. Nilamsari WP, Rizqi MF, Regina NO, Wulaningrum PA, Fatmawati U. Adverse drug reaction and its management in tuberculosis patients with multidrug resistance: a retrospective study. *J Basic Clin Physiol Pharmacol*. 2021;32(4):783–787.
12. Salem C Ben, Slim R, Fathallah N, Hmouda H. Drug-induced hyperuricaemia and gout. *Rheumatol (United Kingdom)*. 2017;56(5):679–688.
13. Fardin RO. Pengaruh pemberian alopurinol tablet dan probenesid tablet terhadap kadar asam urat darah kelinci yang diinduksi kalium oksonat. 2019;16(1).
14. Susilo R. Translation and validation of the Indonesian version of the adverse drug reaction severity level instruments in colorectal cancer patients [Internet] *J Multidiscip Healthc*; 2022:1153–1161. Available from: https://www.dovepress.com/translation-and-validation-of-the-indonesian-version-of-the-adverse-dr-peer-reviewed-fulltext-article-JMDH.
15. Lakhani P, Singh D, Barua S, et al. An observational study to analyze predisposing factors, causality, severity and preventability of adverse drug reactions among multidrug resistant tuberculosis patients treated under RNTCP program in Northern India. *Int J Res Med Sci*. 2019;7(3):687.
16. Dela AI, Tank NKD, Singh AP, Piparva KG. Adverse drug reactions and treatment outcome analysis of DOTS-plus therapy of MDR-TB patients at district tuberculosis centre: a four year retrospective study. *Lung India*. 2017;34(6):522–526.
17. World Health Organization (WHO). *Consolidated Guidelines on Tuberculosis Treatment*. Who; 2020:99.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Original article

Adverse drug reactions due to linezolid in the programmatic management of drug-resistant tuberculosis in India: A retrospective multicenter study

Gyanshankar Mishra [a,*], *Jan-Willem Alffenaar* [b,c,d], *Radha Munje* [a], *Sadaf Khateeb* [a]

[a] *Department of Respiratory Medicine, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India*
[b] *Sydney Institute for Infectious Diseases, University of Sydney, Sydney, NSW, Australia*
[c] *Faculty of Medicine and Health, School of Pharmacy, University of Sydney, Sydney, NSW, Australia*
[d] *Westmead Hospital, Sydney, NSW, Australia*

ARTICLE INFO

Article history:
Received 29 December 2022
Received in revised form
30 March 2023
Accepted 5 April 2023
Available online 10 April 2023

Keywords:
Linezolid
Peripheral neuropathy
ADR
Anti-TB treatment
MDR-TB

ABSTRACT

Background: Monitoring and managing adverse drug reactions (ADR) are critical for treating drug-resistant tuberculosis (TB).
Objective: To study symptomatic, linezolid-attributable ADRs in TB patients initiated on all oral longer bedaquiline-based treatment regime for multidrug-resistant/rifampicin-resistant (MDR/RR)-TB under programmatic conditions.
Methods: It was a multicenter, retrospective study of people with MDR/RR-TB in nine TB units in Nagpur, India, from March 2020 to April 2022.
Results: The study consisted of a sample size of 106 individuals with multidrug-resistant and rifampicin-resistant tuberculosis out of a total of 110 individuals with the disease. Of these, 45 (42.45%) experienced linezolid ADRs, with an incidence of 11.37 cases per 1000 person-weeks. These patients were significantly younger (31.24 ± 11.13 years) and more likely to be female (27, 50%) than those without ADRs. ADR severity was mild in 20 (44.45%), moderate in 15 (33.33%), and severe in 10 (22.22%) patients. The most common ADR was peripheral neuropathy (42, 93.33%), followed by lactic acidosis (3, 6.67%), anemia (2, 4.44%), and optic neuritis (2, 4.44%). Dosing was reduced in 17 (37.78%) patients, and linezolid was withdrawn entirely in 19 (42.22%) patients. Only 9 (20%) patients continued linezolid

Abbreviations: TB, Tuberculosis; MDR-TB, Multidrug resistant tuberculosis; RR-TB, Rifampicin-resistant tuberculosis; ADR, Adverse drug reaction; WHO, World Health Organisation; TU, Tuberculosis Unit; PMDT, Programmatic Management of Drug-Resistant TB in India; LPA, Line probe assay; LCDST, Liquid culture drug susceptibility testing; HIV, human immunodeficiency virus; MNSI, Michigan Neuropathy Screening Instrument; NSAIDs, Non-steroidal anti-inflammatory drugs; CYP450, Cytochromes P450; FM-100 hue test, Farnsworth-Munsell 100-hue test; mg/kgBW/day, milligrams per kilogram of body weight per day; DNA, Deoxyribonucleic acid; DECODE, DElpazolid Dose-finding and COmbination DEvelopment; PanACEA, Pan African Consortium for the Evaluation of Antituberculosis Antibiotics; SUDOCU, Sutezolid Dose-finding and Combination Evaluation; aDSM, active TB drug-safety monitoring and management; ADR-OPDs, Adverse Drug Reaction-Outpatient Departments; TDM, Therapeutic drug monitoring.

* *Corresponding author*. Department of Respiratory Medicine, Indira Gandhi Government Medical College, Nagpur, Maharashtra, 440018, India. Tel: +91-9850349074.
E-mail address: gpmishra81@gmail.com (G. Mishra).

https://doi.org/10.1016/j.ijtb.2023.04.006

unmodified. For mild to moderate linezolid-associated symptomatic peripheral neuropathy, symptom management with or without dose reduction is an effective strategy; however, immediate linezolid withdrawal is necessary in severe or life-threatening peripheral neuropathy cases. After a mean follow-up of 41 ± 21.33 weeks, ADR symptoms resolved completely in 4 (6.67%) patients and decreased in 42 (93.33%) patients.

Conclusion: Linezolid ADRs, often neuropathy, frequently occur in patients on an all-oral bedaquiline-based treatment regime for MDR/RR-TB. Women and younger patients are more likely to experience these ADRs, usually mild to moderate in severity. Management of symptomatic linezolid-associated peripheral neuropathy should be based on ADR severity. These ADRs often affect linezolid dosing, so it is important to identify and manage them early.

1. Introduction

The growing incidence and diffusion of drug-resistant tuberculosis (TB) presents an enormous community health issue, imposing formidable challenges worldwide. Among these, multidrug-resistant TB (MDR-TB) and rifampicin-resistant TB (RR-TB) represent major hurdles to achieving successful treatment outcomes, requiring second line anti-TB drugs to overcome therapeutic challenges.[1] Vigilant monitoring of adverse drug reactions (ADRs) is paramount to effectively managing drug-resistant TB. Inadequate management of ADRs can potentially result in unfavorable treatment outcomes. Linezolid, a group A second line anti-TB agent according to the World Health Organisation's (WHO) drug classification system, is extensively employed to treat MDR/RR-TB. However, there is a dearth of programmatic treatment data pertaining to linezolid-attributable ADRs in India.[2] In view of its pivotal role in the novel, abbreviated treatment protocols for drug-resistant TB, such as bedaquiline-pretomanid-linezolid and other recommended therapeutic regimens, the prompt detection and proactive management of potentially severe ADRs associated with linezolid in programmatic settings are indispensable.[3]

We aimed to explore the ADRs of linezolid, which culminated in symptomatic manifestations among individuals receiving a bedaquiline-based, all-oral, longer regimen to treat MDR/RR-TB under programmatic conditions.

2. Material and methods

The current investigation was a retrospective, multicenter study that aimed to examine patients diagnosed with multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB), and enrolled in the Programmatic Management of Drug-Resistant TB in India (PMDT). Nine distinct TB units (TUs) located in Nagpur, India, were selected for this study, comprising eight urban TUs and one rural TU. Our study involved an exhaustive analysis of programmatic materials pertaining to people with MDR/RR-TB who received the all-oral longer bedaquiline-based treatment regime for their disease from March 2020 to April 2022. Further, we specifically examined patients who were administered at least one dose of linezolid, regardless of their current treatment status or outcomes.

Variables such as age, gender, body weight, comorbidities, type of TB (pulmonary/extrapulmonary), duration of anti-TB treatment, and linezolid dose were meticulously analyzed and compared between patients who experienced symptomatic ADRs attributed to linezolid and those who did not. Additionally, we evaluated the type and severity of ADRs, the duration between the administration of linezolid and reporting of ADRs, the post-ADR follow-up period, and the progression of ADR symptoms. These endeavors aimed to elucidate the factors influencing the occurrence of linezolid-attributable symptomatic ADRs in individuals with MDR/RR-TB.

2.1. *Anti-TB treatment regime*[4]

The treatment protocol employed involved the administration of levofloxacin, linezolid, clofazimine, and cycloserine over a span of 18–20 months. For the initial six months, bedaquiline was concurrently administered, while linezolid was prescribed at a daily dosage of 600 mg for patients weighing over 30 kg and 300 mg per day for those whose weight ranged from 16 to 29 kg. Subsequently, the administered dose of linezolid was diminished to a solitary intake of 300 mg per day. Additionally, individuals with a body weight greater than 30 kg were prescribed pyridoxine at a dose of 100 mg once per day, while those weighing between 16 and 29 kg were administered 50 mg of the drug on a daily basis.

Before commencing this study, we obtained approval from the institutional ethical committee (Approval no. IGGMC/Pharmacology/IEC/1023-24/2022). Ascertaining that the current study was retrospective, the obligatory stipulation of obtaining informed consent was exempted.

We defined symptomatic ADRs attributable to linezolid as any ADR reported by the patient known to be caused by linezolid.

ADR severity was evaluated based on its effect on the patient's functioning and categorized as mild, moderate, severe, or life-threatening. Mild ADRs did not significantly affect normal functioning, while moderate ADRs caused some impairment but were not hazardous to health. Severe ADRs

significantly impaired or incapacitated patient functioning, and life-threatening ADRs required hospitalization and could cause death if left untreated.[5] To evaluate the ADRs causality, we employed the Naranjo algorithmic scale as our evaluation method.[6]

3. Statistical analysis

The statistical analysis of the study involved using SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) to carry out computations. The categorization of variables of a categorical nature was effectuated through numerical representation and proportional assessments, whereas continuous variables were articulated in terms of their mean values and standard deviations. The analysis of patients who experienced symptomatic ADRs owing to linezolid and those who did not was achieved through an independent *t*-test. Additionally, the proportions of these groups were contrasted via the application of the Chi-square test. In conformity with the highest standards of scientific rigor, a stringent criterion for statistical significance was established, where the attainment of a p-value of less than 0.05 was deemed indispensable. To ascertain the risk of linezolid-related symptomatic ADRs, the authors implemented the incidence density, which takes into account the duration of drug exposure. The computation of the incidence density rate was conducted via the division of the cumulative count of new ADR cases by the combined number of weeks of observation among the assemblage of people with MDR/RR-TB. By implementing this method, the authors could accurately evaluate the likelihood of linezolid-induced symptomatic ADRs in the population under study.

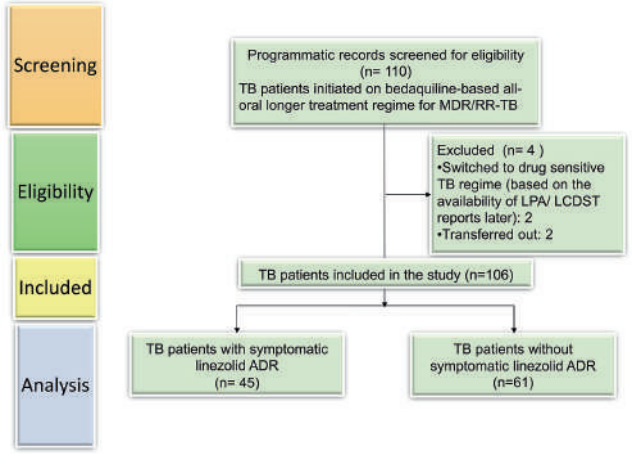


Fig. 1 – Flow chart of the study for determining symptomatic linezolid-attributable ADRs in TB patients initiated on bedaquiline-based all-oral longer treatment regime for MDR/RR-TB under PMDT in India. ADRs: Adverse drug reactions; TB: Tuberculosis; MDR/RR: Multidrug-resistant/ Rifampicin-resistant; PMDT: Programmatic management of drug-resistant TB in India; LPA: Line probe assay; LCDST: Liquid culture drug susceptibility testing.

4. Results

Of the 110 people with MDR/RR-TB screened for the programmatic treatment regime, 106 were eligible for study inclusion and followed for 3959 person-weeks. Of these, 45 patients (42.45%) reported symptomatic linezolid ADRs with an incidence density of 11.37 persons per 1000 person-weeks of observation (Fig. 1).

Table 1 presents the patient characteristics for those with and without symptomatic linezolid ADRs. Patients reporting linezolid ADRs were significantly younger (31.24 ± 11.13 years vs. 38.15 ± 15.42 years ($p = 0.0121$)) with a female preponderance (27 (50%) vs. 23 (37.71%) ($p = 0.0237$)). There existed no noteworthy dissimilarities in weight distribution, TB type, co-existing health conditions, anti-TB treatment duration, or linezolid dosage based on weight between patients who exhibited linezolid-attributable symptomatic ADRs and those who did not.

Table 2 presents the characteristics of symptomatic linezolid ADRs. All ADRs were classified as "probable" according to causality assessment. Following the administration of the treatment, the average period for the onset of ADRs was approximately 16 ± 10.53 weeks, with a typical monitoring phase of about 33 ± 21 weeks. The frequency of ADR occurrence was markedly higher among 40 individuals (comprising 88.89% of the cohort) receiving a daily dose of 600 mg, compared to a mere 5 patients (11.11%) who reported ADRs upon dose reduction to 300 mg once per day following 24 weeks, which was the standard interval.

In the study, peripheral neuropathy was the most common symptomatic linezolid ADR, reported by 42 (93.33%) patients. The onset of symptoms occurred between 4 and 48 weeks, with a mean duration of 16.76 ± 10.41 weeks and included burning sensations in palms and soles and difficulty with walking. The mean post-ADR treatment follow-up duration was 41 ± 21.33 weeks. Partial symptomatic relief was observed in 41 (95.35%) patients, while a complete resolution was observed in 2 (4.65%) patients. Fig. 2 illustrates the impact of the severity of linezolid-associated peripheral neuropathy on the modification of linezolid dosage.

Lactic acidosis, anemia, and optic neuritis were other infrequent linezolid ADRs noted in seven patients, among whom linezolid was stopped in six and continued at a lower dose in one (Table 2).

5. Discussion

A considerable proportion (42.45%) of patients receiving all-oral bedaquiline therapy for MDR/RR-TB experienced symptomatic linezolid ADRs, underscoring linezolid's substantial limitation in programmatic use.

There are several risk factors that may impact the onset of peripheral neuropathy as a complication of anti-TB treatment, including but not limited to HIV infection, chronic alcoholism, malnutrition, co-infection, diabetes, kidney impairment, pregnancy, and lactation. These factors are well-established and have been implicated in the etiology of this debilitating condition.[7] Upon exploring the data, we observed that young

Table 1 – Comparison of demographic and clinical characteristics in TB patients with and without linezolid-attributable ADRs in the bedaquiline-based all-oral longer treatment regime for MDR/RR-TB under PMDT.

Serial number	Parameters	TB patients developing symptomatic linezolid ADRs (n = 45)	TB patients who did not develop symptomatic linezolid ADRs (n = 61)	P value
1	Age [Mean ± SD]	31.24 ± 11.13 years	38.15 ± 15.42 years	0.0121
2	Gender [number (proportion)]	Males:18 (40%) Females:27 (60%)	Males: 38 (62.30%) Females:23 (37.71%)	0.0237
3	Weight [Mean ± SD]	42 ± 10.49 kg	44.1 kg ± 12.16 kg	0.3542
4	Type of Tuberculosis [number (proportion)]	Pulmonary:36 (80%) Extrapulmonary: 9 (20%)	Pulmonary: 51 (83.61%) Extrapulmonary: 10 (16.39%)	0.6336
5	Presence of comorbidity [number (proportion)]	8 (20%) PLHIV: 2 Diabetes: 5 Alcoholism: 1	5 (8.2%) PLHIV: 2 Diabetes: 3	0.0776
6	Duration of Anti-TB Treatment [Mean ± SD]	57 ± 21.01 weeks	53 ± 33.77 weeks	0.4825
7	Linezolid dose [Mean ± SD]	15 ± 3.21 mg/kg	14 ± 3.12 mg/kg	0.1102

TB: Tuberculosis; ADRs: Adverse drug reactions; MDR/RR: Multidrug-resistant/Rifampicin-resistant; PMDT: Programmatic management of drug-resistant TB in India; PLHIV: People living with HIV.

Table 2 – Characteristics of symptomatic linezolid-attributable ADRs.

Serial Number	Parameter	Observation
1	TB patients developing symptomatic linezolid ADR events [number (proportion)]	
	Peripheral Neuropathy	42 (93.33%)
	Lactic acidosis	3 (6.67%)
	Anaemia	2 (4.44%)
	Optic neuritis	2 (4.44%)
2	ADR Severity	Mild: 20 (44.45%) Moderate: 15 (33.33%) Severe: 10 (22.22%) Life-threatening: 0 (0%)
3	Duration to develop ADR after treatment initiation [Mean ± SD]	16 ± 10.53 weeks
4	Impact on Linezolid Dosing[a] [number (proportion)]	
	Linezolid dose unmodified	9 (20%)
	Linezolid dose reduced	17 (37.78%)
	Linezolid stopped	19 (42.22%)
5	Post-ADR follow-up duration	41 ± 21.33 weeks
6	ADR symptom progression	
	Complete symptomatic resolution of ADR	3 (2 (peripheral neuropathy) 1 (lactic acidosis)) (6.67%)
	ADR symptoms decreased	42 (93.33%)
	Persistence or worsening of ADR symptoms	0 (0%)

ADRs: Adverse drug reactions; TB: Tuberculosis.

[a] The dose of linezolid was 600 mg per day in 42 TB patients, whereas it was 300 mg in three TB patients at the time of ADR reporting by the patients.

age exhibits itself as a notable risk factor for linezolid-attributable symptomatic ADRs in conjunction with the female gender. Wasserman et al. conducted a study that suggested that the susceptibility of females with TB to linezolid ADRs could be attributed to variations in immunological and hormonal factors, rather than differences in pharmacokinetics.[8] Lower lean body mass and altered drug metabolism rates may also contribute as additional factors.

Currently, no established treatment for peripheral neuropathy caused by linezolid is available, and management aims to provide symptomatic relief. The detrimental effects of this condition on patients highlight the importance of preventing it when feasible. The Michigan Neuropathy Screening Instrument (MNSI), as a simple and easily applicable screening tool, represents a valuable asset for the prompt identification of linezolid-induced neuropathy in programmatic environments.[9]

The potential for cycloserine and linezolid-induced peripheral neuropathy looms as a significant concern within the current therapeutic regimen. As a pyridoxine antagonist, cycloserine fosters renal elimination of pyridoxine and engenders concomitant pyridoxine deficiency.[10] Consequently,

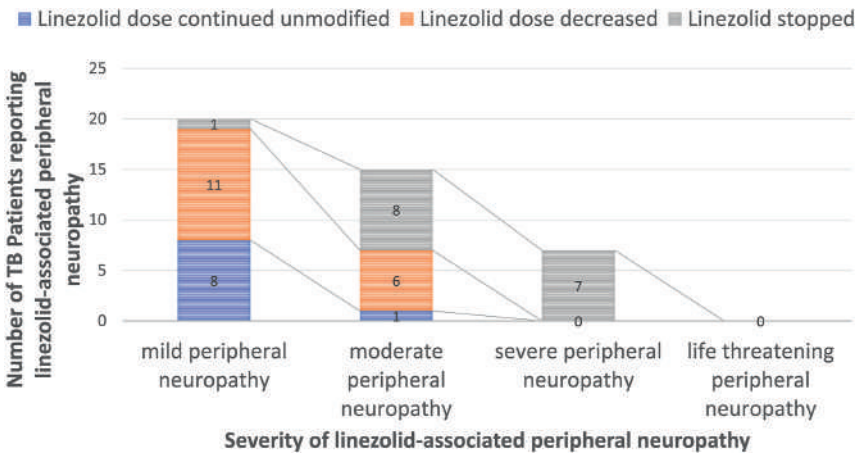


Fig. 2 – Linezolid dose modification as per the severity of linezolid-associated peripheral neuropathy in TB patients initiated on bedaquiline-based all-oral longer treatment regime for MDR/RR-TB under PMDT. TB: Tuberculosis; MDR/RR: Multidrug-resistant/Rifampicin-resistant; PMDT: Programmatic management of drug-resistant TB in India.

the administration of 100 mg per day of pyridoxine is a standard recommended component of routine programmatic care.[11] In response to the initial signs of peripheral neuropathy, the pyridoxine dosage is immediately elevated to 200 mg per day,[10] as this approach has demonstrated efficacy in mitigating symptoms.[8] The use of cycloserine may rarely result in vitamin B12 deficiency, while linezolid-containing anti-tuberculosis treatment has also been linked to deficiencies in both vitamin B1 and B12, which may contribute to the development of peripheral neuropathy.[12,13]

Linezolid disrupts mitochondrial protein synthesis and respiratory chain function, leading to mitochondrial dysfunction in metabolically active cells and resulting in ADRs such as lactic acidosis and peripheral neuropathy.[14]

Linezolid toxicity exhibits dose and duration-dependent characteristics.[15] Previous studies have demonstrated that ADRs, particularly peripheral neuropathy, were reported after a treatment duration of 8 weeks.[16] In our study, peripheral neuropathy onset occurred at an average of 16 ± 10.53 weeks after initiating linezolid treatment, with the majority (88.89%) receiving the WHO-recommended daily dose of 600 mg once daily. These findings emphasize the crucial need for early and routine monitoring of linezolid-associated ADRs at this dosage.

In managing linezolid-induced peripheral neuropathy, symptomatic relief can be achieved using vitamin B-complex, coenzyme Q, pregabalin, gabapentin, and NSAIDs. However, treatment may be complicated by drug interactions with bedaquiline. While tricyclic antidepressants, such as amitriptyline or nortriptyline, may effectively manage peripheral neuropathy, their use is associated with an elevated risk of bedaquiline-related cardiac rhythm disturbances and the potential for serotonin syndrome in patients concomitantly receiving linezolid.[17] In the context of bedaquiline therapy, carbamazepine, a potent CYP450 inducer, may decrease bedaquiline levels, potentially reducing its therapeutic efficacy. Non-pharmacologic measures, such as physiotherapy techniques and ice packs, may help alleviate symptoms of the condition.

Our findings (Fig. 2) suggest that a severity-based approach (Table 3) is preferable to discontinuing linezolid at the onset of peripheral neuropathy, as recommended by some authors. For mild to moderate neuropathy, we propose attempting symptomatic management for a maximum of seven days, either with or without reducing the linezolid dose to 300 mg. Given its essential role in the therapeutic regimen for MDR/RR-TB, it is imperative to minimize the discontinuation of linezolid, if at all possible.[18] In cases where symptomatic management does not improve symptoms within seven days, discontinuation of linezolid is recommended. Severe and life-threatening neuropathy warrants permanent discontinuation of linezolid. It is noteworthy that some cases of linezolid-associated neuropathy may be irreversible.[19] To avoid permanent and debilitating ADRs, prompt withdrawal of linezolid is imperative. Our study revealed that 42.22% of TB patients required permanent discontinuation of linezolid due to ADRs. Therefore, timely and appropriate management, including withdrawal when necessary, is critical for effectively managing linezolid-associated neuropathy.

Jaspard et al. reported irreversible linezolid-associated peripheral neuropathy in 78% of TB patients at one year of post-treatment follow-up despite linezolid withdrawal. During the Nix-TB clinical trial, a combination therapy comprising bedaquiline, pretomanid, and a high daily dose of linezolid (1200 mg) resulted in frequent incidences of neuropathy associated with linezolid use, although with some amelioration over an extended follow-up period of 24 months.[3]

In our study, treatment with linezolid was immediately discontinued in three (6.67%) patients who experienced symptoms consistent with lactic acidosis. Although relatively rare, linezolid-associated lactic acidosis is a serious ADR associated with a high mortality rate of 25.5%.[20] Failure to recognize this condition may result in misdiagnosis as other common side effects of anti-TB drugs, such as gastritis or hepatitis.

In our study, two (4.44%) patients developed optic neuritis related to linezolid treatment. The current research outcomes are in accordance with those of Mehta et al., who revealed a

Table 3 – A suggested management strategy for linezolid-associated peripheral neuropathy based on ADR severity in the WHO-recommended bedaquiline-based all-oral longer regime in programmatic management of drug-resistant TB in India.

Serial number	Clinical symptoms[5]	The severity of linezolid-associated peripheral neuropathy[5]	Suggested management strategy
1	Mild numbness & weakness in hands & feet	Mild	1. Symptomatic management with the continuation of linezolid with the unmodified dose (600 mg) for seven days. 2. If there is no symptomatic relief, then the linezolid dose reduction (300 mg od) for seven days and re-assess after seven days. If there is symptomatic relief, continue with the reduced linezolid dose for the rest of the duration of the treatment regime. 3. If there is no symptomatic relief, then withdraw linezolid permanently from the regime.
2	Prickling, stabbing, burning, or tingling sensation along with a gradual increase in numbness & weakness	Moderate	1. Dose reduction (300 mg od) for seven days with symptomatic management and re-assess after seven days. If there is symptomatic relief, continue with the reduced linezolid dose for the rest of the duration of the treatment regime. 2. If there is no symptomatic relief, then withdraw linezolid permanently from the regime.
3	Symptoms of moderate neuropathy along with extreme sensitivity to touch	Severe	1. Stop linezolid immediately. 2. Symptomatic management.
4	Muscle weakness with lack of coordination and difficulty in balancing. There may be poor control of bowel & bladder movements.	Potentially Life-threatening	1. Stop linezolid immediately. 2. Symptomatic management. 3. Hospitalization.

frequency of 5.81% among Mumbai, India's drug-resistant TB patients, and Jaspard et al., who documented optic neuritis in 24.56% of TB patients, of which 35.71% of cases were asymptomatic.[18,21]

The possibility of linezolid-associated optic neuritis should be considered, even without ocular symptoms. Clinical features such as blue-yellow colour blindness and an increased blind spot on perimetry are typical. As such, it is of utmost importance to conduct meticulous assessments for any possible deviations in color vision, particularly in individuals with blue-yellow deficiencies that may not be easily detectable through standard color vision charts such as the Ishihara chart. To precisely identify such deficiencies, the F-M100 hue test is strongly recommended. Furthermore, a fundus examination can offer valuable information regarding any incidents of papillary edema or hyperemia of the optic nerve.[18,22] Visual impairment is a common symptom of linezolid-associated optic neuritis. A comprehensive evaluation should include colour vision testing with the FM-100 hue test, perimetry, fundus examination, and visual acuity testing. Mehta et al. reported that administering oral prednisolone therapy (starting at a daily dose of 40 mg and gradually tapered by 10 mg per week) and discontinuing linezolid can result in a favorable outcome.[21]

In our study, anemia was observed in two patients (4.44%) who were receiving linezolid for the management of MDR-TB, with discontinuation of the drug in one of the patients. A frequently observed hematological adverse effect of linezolid therapy is anemia, which is more commonly seen in patients with a body weight below 54 kg or those administered a higher dose of linezolid exceeding 11 mg/kgBW/day.[23] Both patients with anemia in our study had one or both of these risk factors. According to the PMDT program, it is recommended to carry out frequent monitoring of hemoglobin and complete blood cell count on a monthly basis during linezolid therapy, administered once daily at a dose of 600 mg, followed by quarterly check-ups after reducing the dose to 300 mg once daily. This proactive approach is aimed at preventing the potential occurrence of linezolid-induced anemia.[4]

Linezolid can have deleterious effects on mitochondria, the cellular organelles responsible for energy production. Recent studies have identified specific genetic markers that may increase an individual's risk of developing linezolid-induced mitochondrial toxicity. The possession of mitochondrial haplogroup U is linked to an augmented vulnerability to linezolid toxicity, with this marker being identified in 15% of Indians and 11% of native Europeans.[24,25] Conversely, mitochondrial DNA

haplogroup H has been associated with a lower incidence of linezolid toxicity, and this haplogroup is more abundant in European populations compared to other populations, such as the Indian population.[19,24] These genetic risk factors may guide tailored treatment approaches to minimize linezolid's adverse effects in susceptible individuals.

Linezolid stands as the only oxazolidinone currently recommended for drug-resistant TB treatment. Nevertheless, promising anti-TB agents such as sutezolid and tedizolid have emerged, and delpazolid is speculated to exhibit a more benign toxicity profile.[26] The commencement of the DElpazolid dose-finding and Combination Development (DECODE) clinical trial under the auspices of PanACEA, an alliance dedicated to assessing anti-tuberculosis antibiotics in Africa, represents a significant undertaking to evaluate the safety and efficacy of delpazolid as a co-administered therapy with bedaquiline, delamanid, and moxifloxacin for the treatment of tuberculosis resistant to standard drugs. This initiative seeks to advance the fight against TB by exploring new treatment possibilities. Patients enrolled in this randomized trial will receive a four-month regimen of the four drugs, and their outcomes will be compared to those of SUDOCU, a similar study examining the combination of sutezolid, bedaquiline, delamanid, and moxifloxacin. Consequently, the study designs of DECODE and SUDOCU enable a head-to-head evaluation of delpazolid and sutezolid's effectiveness and toxicity in treating drug-resistant TB.[27] Tedizolid, characterized by lower minimum inhibitory concentration than linezolid, could also emerge as a viable and safer alternative for drug-resistant TB treatment.[28]

The introduction of newer, potentially less toxic oxazolidinones for treating drug-resistant TB is eagerly anticipated, but until they become routinely available, proactive measures must be taken to minimize linezolid-associated ADRs. The PMDT initiative puts forth a robust stance on monitoring and managing the safety of TB drugs through active measures, referred to as aDSM or "active TB drug-safety monitoring and management." This includes the utilization of essential instruments, such as an aDSM treatment initiation form specifically designed for every patient diagnosed with MDR/RR-TB, an aDSM review form to assess severe adverse events, and the incorporation of an ADR module in Nikshay, a web-based application tailored to TB management.[4,5] However, successful aDSM implementation requires dedicated ADR-OPDs for TB, increased human resource allocation, and infrastructure upgrades, which must be prioritized in programmatic conditions.

Linezolid, an important treatment option for MDR-TB, poses a risk of ADRs. To minimize this risk, it is recommended that linezolid be reserved for patients known to be sensitive to the drug. However, the absence of a rapid drug-susceptibility testing method for linezolid recommended by the WHO necessitates the use of traditional testing methods to determine a patient's sensitivity, which can lead to toxicity in some patients before culture results are available.[29] Therefore, the development of a rapid linezolid susceptibility test is urgently needed. Moreover, conducting genetic testing on patients prior to treatment can be a promising strategy for identifying individuals who are susceptible to severe ADRs induced by linezolid therapy.

The emergence of ADRs with linezolid treatment, particularly peripheral neuropathy, calls for measures to manage and prevent these occurrences. Therapeutic drug monitoring (TDM) has emerged as a promising solution to address the potential challenges associated with drug efficacy and toxicity, prompting its widespread consideration and discussion in various medical settings.[17] Studies have demonstrated that elevated linezolid trough concentrations exceeding 2 mg/L correlate with an increased risk of neuropathy. Despite this knowledge, the use of TDM for linezolid is yet to be widely adopted, and access to testing may be limited in certain regions.[30] In light of these challenges, point-of-care saliva tests have emerged as a viable option for screening high drug concentrations at the initiation of linezolid therapy. This approach provides an easy and cost-effective means to identify patients at risk for ADRs, enabling early dose adjustments to mitigate ADR occurrence.[31]

The present study contributes towards a more comprehensive understanding of the incidence and consequences of symptomatic ADRs linked to linezolid administration in individuals suffering from MDR/RR-TB. However, the study only examined patient-reported symptomatic ADRs, potentially overlooking asymptomatic ADRs that require additional tests for detection. Moreover, being retrospective, it might have suffered from underreporting or recall bias. Nevertheless, the study highlights the need for further research to address the management of asymptomatic ADRs and personalize dosing and genetic factors to improve linezolid's efficacy.

6. Conclusion

Linezolid is an integral component of the bedaquiline-based all-oral longer treatment regime for MDR/RR-TB, which is currently the frontline option for managing these patients. However, our study has highlighted the significant risk of linezolid-associated symptomatic ADRs, particularly peripheral neuropathy, in people with MDR/RR-TB. Females and younger cohorts are more susceptible to these ADRs. Effective management of ADRs requires meticulous monitoring and timely intervention. Systematic screening, meticulous record-keeping, and comprehensive reporting mechanisms for ADRs are essential to promote optimal patient safety. Further research is needed to develop effective strategies for managing linezolid-related symptomatic ADRs, including personalized dosing and genetic testing. Adopting a proactive stance in managing ADRs for optimally utilizing linezolid in the fight against drug-resistant TB within India's PMDT program is crucial.

Financial support and sponsorship

This was an investigator-initiated non-funded study.

Author contributions

Dr. Gyanshankar Mishra worked on conceptualizing the study work, study design, submission of the IEC proposal, collecting, compiling, analyzing data, and drafting the manuscript along with intellectual inputs. Professor Johannes (Jan-Willem)

Alffenaar contributed to the study design, critical evaluation of the results, and manuscript draft and provided intellectual inputs. Dr. Radha Munje provided intellectual input. Dr. Sadaf Khateeb provided help in gathering and providing individual person-related information from the NTEP/PMDT records and reliable inputs in the manuscript pertaining to individual person-related issues in the field. Dr. Gyanshankar Mishra is the guarantor of the paper.

Conflicts of interest

The authors have none to declare.

Acknowledgment

The authors are thankful to Dr. Shilpa Jichkar (City TB officer, Nagpur), Dr. Anil Sontakke (Professor, Respiratory Medicine, NKPSIMS, Nagpur), Dr. Aparna Chayande, (Medical Officer, PMDT Site, NKPSIMS, Nagpur) & Mr. Uttam Madhumatke (PMDT Supervisor, Nagpur Municipal Corporation) for support and help in access to PMDT treatment records for the research study.

REFERENCES

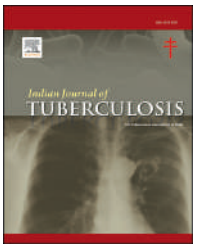
1. World Health Organization. *Global Tuberculosis Report 2022*. 2022. Geneva, Switzerland.
2. Bolhuis MS, Akkerman OW, Sturkenboom MGG, et al. Linezolid-based regimens for multidrug-resistant tuberculosis (TB): a systematic review to establish or revise the current recommended dose for TB treatment. *Clin Infect Dis*. 2018 Nov 28;67(suppl_3):S327–S335.
3. Mishra G. Nix-TB and ZeNix trials: paving the way for shorter regimens for drug-resistant tuberculosis. *Asian Pac J Tropical Med*. 2021 Oct 29;14(10):431–432.
4. Central TB Division India. *Guidelines for Programmatic Management of Drug Resistant Tuberculosis in India 2021 [Internet]*. Central TB Dvision, Ministry of Health and Family Welfare India; 2021:1–293. Available from: https://tbcindia.gov.in/showfile.php?lid=3590.
5. Central Tuberculosis Division. *Ready Reckoner for Medical Officers: Adverse Drug Reactions Associated with Ready Reckoner for Medical Officers, Identification & Management Anti-Tubercular Drugs [Internet]*; 2020 [cited 2022 Jul 19]. Available from: https://drive.google.com/file/d/1otkjE1G4yOIlhAFKu4SPNWficnOhfni_/view.
6. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther*. 1981;30(2):239–245.
7. World Health Organization (WHO). *WHO Operational Handbook on Tuberculosis (Module 1 – Prevention): Tuberculosis Preventive Treatment*. 2020:1–140.
8. Abdelwahab MT, Wasserman S, Brust JCM, et al. Linezolid population pharmacokinetics in South African adults with drug-resistant tuberculosis [cited 2022 Nov 17] *Antimicrob Agents Chemother [Internet]*. 2021 Dec 1;65(12), e0138121. Available from: https://pubmed.ncbi.nlm.nih.gov/34543098/.
9. Zhang P, Li W, Liu M, et al. Linezolid-associated neuropathy in patients with MDR/XDR tuberculosis in shenzhen, China. *Infect Drug Resist [Internet]*. 2022 May;15:2617–2624 [cited 2022 Jun 2]; Available from: https://pubmed.ncbi.nlm.nih.gov/35634579/.
10. Shin SS, Hyson AM, Castañeda C, et al. Peripheral neuropathy associated with treatment for multidrug-resistant tuberculosis. *Int J Tubercul Lung Dis*. 2003 Apr;7(4):347–353.
11. Alffenaar JWC, Stocker SL, Forsman LD, et al. Clinical standards for the dosing and management of TB drugs. *Int J Tubercul Lung Dis*. 2022 Jun 1;26(6):483–499.
12. Minardi ML, Fato I, di Gennaro F, et al. Common and rare hematological manifestations and adverse drug events during treatment of active TB: a state of art [cited 2022 Oct 22] *Microorganisms [Internet]*. 2021 Jul 1;9(7):1–15. Available from:/pmc/articles/PMC8304680/.
13. Shvets OM, Shevchenko OS, Todoriko LD, et al. Changes of vitamins B1, B12 as predictors of peripheral polyneuropathy in pulmonary tuberculosis patients. *Arch Balk Med Union*. 2020;55(1):113–120. https://doi.org/10.31688/ABMU.2020.55.1.13.
14. Abou Hassan OK, Karnib M, El-Khoury R, Nemer G, Ahdab-Barmada M, BouKhalil P. Linezolid toxicity and mitochondrial susceptibility: a novel neurological complication in a Lebanese patient. *Front Pharmacol*. 2016 Sep 20;7(SEP):325.
15. Nuermberger E. Evolving strategies for dose optimization of linezolid for treatment of tuberculosis. *Int J Tubercul Lung Dis*. 2016 Dec 1;20(12):S48–S51.
16. Tang S, Yao L, Hao X, et al. Efficacy, safety and tolerability of linezolid for the treatment of XDR-TB: a study in China. *Eur Respir J*. 2015 Jan 1;45(1):161–170.
17. McDowell A, Haas M, Seaworth B, et al. Linezolid use for the treatment of multidrug-resistant tuberculosis, TB centers of excellence, United States, 2013–2018. *J Clin Tuberc Other Mycobact Dis*. 2021 Feb 1;22, 100201.
18. Jaspard M, Butel N, el Helali N, et al. Linezolid-associated neurologic adverse events in patients with multidrug-resistant tuberculosis, France [cited 2022 Feb 7] *Emerg Infect Dis [Internet]*. 2020 Aug 1;26(8):1792. Available from:/pmc/articles/PMC7392460/.
19. Mikiashvili L, Kipiani M, Schechter MC, Avaliani Z, Kiria N, Kempker RR. Linezolid use for drug-resistant tuberculosis in Georgia: a retrospective cohort study. *Int J Tubercul Lung Dis*. 2020 Apr 4;24(4):436.
20. Mao Y, Dai D, Jin H, Wang Y. The risk factors of linezolid-induced lactic acidosis: a case report and review. *Medicine*. 2018 Sep 1;97(36).
21. Mehta S, Das M, Laxmeshwar C, Jonckheere S, Thi SS, Isaakidis P. Linezolid-associated optic neuropathy in drug-resistant tuberculosis patients in Mumbai, India [cited 2022 Oct 21] *PLoS One [Internet]*. 2016 Sep 1;11(9). Available from:/pmc/articles/PMC5017632/.
22. Mate K, Mishra G, Munje R. Adverse drug reactions to a daily fixed-dose combination based anti-tuberculosis treatment regime in India' s national tuberculosis elimination programme : a prospective cohort study. *J Clin Diagn Res*. 2022;16(8):OCD14–OCD19.
23. Pratama NYI, Zulkarnain BS, Soedarsono Fatmawati U. Hematological side effect analysis of linezolid in MDR-TB patients with individual therapy. *J Basic Clin Physiol Pharmacol [Internet]*. 2021 Jul 1;32(4):777–781 [cited 2022 Oct 21]; Available from: https://pubmed.ncbi.nlm.nih.gov/34214355/.
24. Garrabou G, Soriano, Pinós T, et al. Influence of mitochondrial genetics on the mitochondrial toxicity of linezolid in blood cells and skin nerve fibers. *Antimicrob Agents Chemother*. 2017 Sep 1;(9):61.
25. Metspalu M, Kivisild T, Metspalu E, et al. Most of the extant mtDNA boundaries in South and Southwest Asia were likely shaped during the initial settlement of Eurasia by anatomically modern humans. *BMC Genet*. 2004 Aug 31;5:26.

26. Yew WW, Chan DP, Chang KC. Does linezolid have a role in shortening treatment of tuberculosis? *Clin Microbiol Infection*. 2019 Sep 1;25(9):1060–1062.
27. Kim JS, Kim YH, Lee SH, et al. Early bactericidal activity of delpazolid (LCB01-0371) in patients with pulmonary tuberculosis. *Antimicrob Agents Chemother*. 2022;66(2):1–11.
28. Aono A, Murase Y, Chikamatsu K, et al. In vitro activity of tedizolid and linezolid against multidrug-resistant Mycobacterium tuberculosis: a comparative study using microdilution broth assay and genomics. *Diagn Microbiol Infect Dis*. 2022 Jul 1;103(3), 115714.
29. Saderi L, Puci M, di Lorenzo B, et al. Rapid diagnosis of XDR and pre-XDR TB: a systematic review of available tools. *Arch Bronconeumol [Internet]*; 2022 Jul [cited 2022 Oct 24]; Available from: https://pubmed.ncbi.nlm.nih.gov/35945071/.
30. Nahid P, Mase SR, Migliori GB, et al. Treatment of drug-resistant tuberculosis an official ATS/CDC/ERS/IDSA clinical practice guideline. *Am J Respir Crit Care Med [Internet]*. 2019 Nov 15;200(10):E93–E142 [cited 2022 Nov 6]. Available from: http://www.atsjournals.org/doi/suppl/10.1164/rccm.201909-1874ST. www.atsjournals.org.500-501;https://www.atsjournals.org/doi/full/10.1164/rccm.v201erratum2.
31. Kim HY, Ruiter E, Jongedijk EM, et al. Saliva-based linezolid monitoring on a mobile UV spectrophotometer. *J Antimicrob Chemother [Internet]*. 2021 Jul 1;76(7):1786–1792 [cited 2022 Nov 6]; Available from: https://pubmed.ncbi.nlm.nih.gov/33734351/.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/



Review article

Update on drug-resistant pulmonary tuberculosis treatment in hemodialysis patients

Jonny Jonny [a,b,*], *Paramitha Dwiputri Saraswati* [a], *Bunga Pinandhita Ramadhani* [a], *Enda Cindylosa Sitepu* [a], *Taufiq Fredrik Pasiak* [b]

[a] *Department of Internal Medicine, Gatot Soebroto Central Army Hospital, DKI Jakarta, Indonesia*
[b] *Faculty of Medicine, Jakarta Veterans National Development University, DKI Jakarta, Indonesia*

ARTICLE INFO

Article history:
Received 18 January 2023
Received in revised form
15 March 2023
Accepted 10 April 2023
Available online 11 April 2023

Keywords:
Drug-Resistant Tuberculosis (DR-TB)
Drug-Related Side Effects
Chronic Kidney Disease
Hemodialysis
WHO Guidelines

ABSTRACT

World Health Organization (WHO) issued the latest recommendations regarding the management of drug-resistant Tuberculosis (TB) in 2022, allowing the replacement of ethambutol (6 months) with linezolid (2 months). This recommendation also introduced a new regimen, namely bedaquiline, pretomanide, linezolid, moxifloxacin (BPaLM) for fluoroquinolone-sensitive patients and bedaquiline, pretomanide, linezolid, (BPaL) for patients insensitive to fluoroquinolone (6–9 months). The latest TB regimen introduced by WHO provides a shorter-course treatment, however not much has been discussed about the impact of this new regimen on chronic kidney disease (CKD) patients, particularly on hemodialysis (HD). The condition of CKD can interfere with the pharmacokinetics of TB medication, thus could reduce effectiveness and increase toxicity. The drugs used on this new regimen are mostly safe for renal impairment patients due to the dominant metabolism in the liver. Particular precaution is given to the administration of linezolid due to increased hematology side effects and bedaquiline with the side effect of QTC interval lengthening and increased risk of arrhythmias. Although this regimen research has not been in many studies in renal failure patients, no significant side effects nor kidney damage evidence was found. This remains to be proven by more research on the patient population with renal failure.

1. Introduction

TB remains a concern in various fields and is one of the leading causes of death due to infection in the world.[1] In 2021 the number of TB cases reported was 9.5 million with a mortality rate of 1.5 million. The coronavirus disease (COVID) pandemic starting in 2020 caused a decrease in TB diagnosis and access to therapy, which led to increased deaths and drug-resistant TB. The WHO report in 2021 showed a 15%

* Corresponding author. Department of Internal Medicine, Gatot Soebroto Central Army Hospital, Abdul Rahman Saleh Raya Street, No. 24, Senen, Central Jakarta, DKI Jakarta, Indonesia. Tel.: +62-81269075002.
E-mail address: Jonny_army@yahoo.com (J. Jonny).
https://doi.org/10.1016/j.ijtb.2023.04.011

decrease in access to drug-resistant TB therapy from 2019 until 202. This could lead to an increased burden and risk of transmission.[2]

TB can be treated and prevented. However, the duration of TB treatment and the medical side effects cause non-compliance in patients, increasing the risk of drug-resistant tuberculosis.[3] Although TB therapy has grown rapidly, antimicrobial resistance remains a concerning problem and threat to health. It is estimated that there are around 500,000 new cases of drug-resistant TB each year, but only 1 in 3 cases receive appropriate therapy.[2]

CKD is also a major problem in the medical field. Patients with CKD are associated with an increased risk of infections, and TB is one of the most frequent ones.[4] The incidence of TB in CKD patients is around 60/100,000 in the United Kingdom (UK) and 19,270/100,000 in China. CKD patients at various stages have a higher risk of TB, with a higher incidence in dialysis patients.[5] In a study in the UK, there was an increased risk of TB in HD patients (85x) followed by peritoneal dialysis (26x) and functioning transplant patients (20x) compared to the general population in the UK.[6] As we know, some drugs are metabolized in the kidneys, thus, renal impairment can affect the metabolism of drugs.

Tuberculosis patients with CKD have a worse prognosis and are more difficult to treat.[7] The condition of CKD can interfere with the pharmacokinetics of TB medications, thereby reducing the effectiveness of treatment and risking more toxicity and side effects.[8] CKD is also one of the factors leading to failure and increased length of treatment. Therefore, it requires drug doses and administration procedures adjustment, particularly in patients under HD.

In 2022, the WHO issued the latest recommendations for managing drug-resistant TB. On the guidelines of short-term oral TB treatment of bedaquiline (Bdq), levofloxacin (Lfx)/moxifloxacin (Mfx), clofazimine (Cfz), ethambutol (E), ethionamide (Eto), isoniazid high doses (Hh) and pyrazinamide (Z) for (4–6 months) followed by Lfx/Mfx, Cfz, E and Z (5 months) allowed replacement of ethambutol (6 months) with linezolid (2 months). This recommendation also introduced a new regimen, namely Bdq, pretomanide (Pto), linezolid (Lnz), Mfx (BPaLM) in fluoroquinolone-sensitive patients (6–9 months) and Bdq/Pto/Lnz (BPaL) in patients who are insensitive to fluoroquinolone (6–9 months).[9,10] Not much has been discussed about the impact of this new regimen of drugs on CKD patients, particularly in HD patients. This literature review aims to explain the impact of resistant TB drugs on CKD patients undergoing HD, treatment procedures, and dose adjustments, focusing on the latest drug-resistant TB regimen.

2. Discussion

2.1. *Hemodialysis*

HD is one of the most commonly used renal replacement therapies (RRT).[18] The principle of dialysis is to alter the solute composition of solution A by exposing it to the second solution (solution B) across a semipermeable membrane. Two processes occur as the solution penetrates the membrane; diffusion and ultrafiltration. Diffusion is the process of transferring a solute due to differences in the concentration of the substance in the blood and dialysate, which passes passively and randomly. In diffusion, the displacement of solutes is longer for those with higher molecular weight. Another process is ultrafiltration, which is the transfer of liquid and its molecules across the membrane due to hydrostatic or osmotic pressure. However, molecules that are larger than the membrane's pores are retained and do not cross the membrane during ultrafiltration.[11]

2.2. *Factors affecting the excretion of drugs in hemodialysis*

Several drugs are metabolized in the kidneys, thus, renal impairment leads to a decrease in the excretion of drugs. Therefore, it is necessary to make adjustments by lowering the dosage and increasing the time interval between the consumption of drugs or a combination of the two.[12] A decrease in the glomerular filtration rate leads to an increase in the drug's half-life, causing an accumulation of drug levels if administered repeatedly and eventually leading to toxic effects. The increase in half-life also needs a more prolonged duration before getting the maximum effect of the drug.[13]

In patients undergoing HD, the excretion of small molecules, including drug molecules, occurs. Factors influencing drug excretion in HD include the type of drug, type of HD, and patient characteristics.[14] The main factor is the amount of drug excreted in the normal kidneys (extra renal elimination fraction). The higher the extra renal elimination fraction, the greater the pharmacokinetic influence of HD.[21] The amount of drug excreted in dialysis devices depends on water-solubility, molecular weight, binding to proteins, and volume distribution.[24] In HD, the excretion of drugs can exceed that in normal kidneys. In normal kidneys, drug reabsorption in the tubules may occur, whereas this process does not happen in HD. Drugs with large molecules are most likely not excreted during HD.[14] In other words, high molecular weight drugs, high bonds with proteins, and the majority of extrarenal excretion will not be affected by the RRT.

The HD technique affects the amount of excreted drugs. The transfer of molecules through the semipermeable membrane occurs through diffusion and ultrafiltration. In HD the ratio between dialysate flow (Q_D) to blood flow (Q_B) determines the duration of the diffusion process. In intermittent HD, it is common to use Q_D higher than the Q_B rate, thus, dialysis efficacy depends on Q_B. The diffusion speed through the dialysis membrane is related to the molecule weight of the drug, surface area, and membrane thickness. Excreted drug levels are also associated with prolonged HD action. The filter material on the membrane also affects the speed of diffusion.[14] The higher the ultrafiltration rate (UFR), blood flow, dialysate flow, and hemofilter permeability, the longer the dialysis time and the higher the excreted drug levels.[16] In addition, administration of the drug adjacent to the HD time leads to a higher influence of drug excretion. Drug administration on HD is ideally taken at the end of the dosing interval. However, in some drugs with high excretion levels in HD, additional doses may be required at the end of HD.[15]

Drug elimination in HD patients is influenced by residual renal function (RRF), whereas drug distribution is related to

the number of plasma proteins and red blood cells.[17] In cases of anemia and hypoalbuminemia, there will be impaired drug distribution. In HD, rapid clearance of drug levels can occur. HD is also a problem in itself due to intermittent administration. Therefore, some suggest that the administration of the drug be carried out after the acquisition of dialysis.[18]

2.3. *Combination of drug-resistant pulmonary tuberculosis treatment in chronic kidney disease on hemodialysis*

Drug-resistant pulmonary tuberculosis (DR TB) is a TB infection that attacks the body caused by drug-resistant mycobacterium tuberculosis bacteria.[19] Currently, the regimen for drug-sensitive TB therapy is 2 months of RHZE followed by 4 months RH. In DR TB, there is resistance to one or more of these types of drugs. There are several types of DR TB, namely; monoresistance TB (resistant to 1 type of first-line drug), polyresistance TB (resistant to more than 1 type of first-line drug but not resistant against isoniazid simultaneously), MDR-TB (resistant to rifampicin and isoniazid simultaneously), pre-XDR TB (MDR-TB compliant and resistant to fluoroquinolone), and XDR TB (meet the criteria of pre-XDR TB and is resistant to at least 1 group a drug Bdq, Lnz, Lfx/Mfx).[20]

Treatment for DR TB takes a longer and greater amount of regimen. Guidelines for rifampicin resistance TB (RR TB) and MDR-TB, according to WHO in 2020 is divided into two, which are short regimen (9–11 months) and long-term regimen (12–20 months) (Fig. 1). A short regimen is given to patients with no history of using the second-line category of TB drugs and not resistant to fluoroquinolone. This short regimen is Bdq/Lfx/Cfz/Z/E/Hh/Eto (4–6 months) followed by Lfx, Cfz, E and Z (5 months).[20] Guidelines by WHO in 2022 allowed the replacement of ethionamide (4–6 months) with linezolid (2 months). WHO also introduced a new regimen of BPaLM for 6–9 months and BPaL for pre-XDR and XDR-TB patients. A long-term regimen is administered to patients who do not meet the criteria of a short regimen. The long-term regimen on WHO 2020 is 3 group A drugs and 2 group B drugs for 18–24 months that can be replaced with group C drugs if an intolerance or resistance occurs in both other groups, with Bdq administered only up to 6 months.[10] Patients with renal impairment, short-term treatment should not be given.[21]

As previously explained, patients with renal impairment will affect the pharmacokinetics of drugs, especially those which are mostly metabolized in the kidneys.[12] Therefore, TB patients with renal impairment should be given dose adjustments on some drugs (Table 1). In addition, patients with kidney disease have a higher risk of side effects associated with administering anti-TB drugs than normal people.[22] Administration of Mfx, Bdq, Cfz, Dlm, Eto/Pto in patients with renal impairment with or without RRT does not need drug adjustment because most of these drugs are metabolized in the liver and are not excreted from the kidneys.[23] However, some drugs such as Mfx, Bdq, and Lnz require strict monitoring due to their side effects that can increase the risk of kidney damage.[22]

Mfx and Bdq have the side effect of lengthening QTc intervals which are at risk of causing heart rhythm disturbances.[24,25] Renal failure patients often have primary heart disease, polypharmacy, and electrolyte changes in the HD process. This can increase the risk of arrhythmia, leading to cardiac arrest in CKD patients.[26] Delamanids are mostly metabolized in the liver and do not need adjustment in renal impairment patients. However, there is not enough data regarding the excretion of this drug in HD or peritoneal dialysis, therefore, this drug is not recommended in patients with severe renal impairment with or without the RRT. Delamanids also have dangerous QTc interval lengthening side effects in HD patients.[27]

The administration of Lnz causes hematological side effects, such as anemia followed by thrombocytopenia and leukopenia. As we know, in patients with renal failure, there is often anemia due to impaired erythropoietin production. This condition can exacerbate hematologic side effects on the administration of Lnz in drug-resistant TB with CKD.[28]

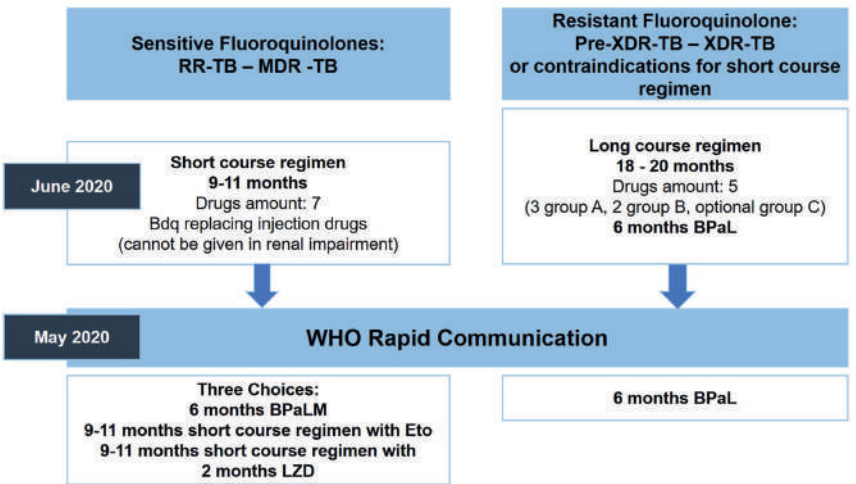


Fig. 1 – Combination of drug-resistant Tuberculosis treatment in renal impairment and regimen changes based on World Health Organization guidelines.

Table 1 – Drug-resistant Tuberculosis medications and dose adjustment in renal impairment patients.

Category	Drug name	Drug dosage	Excretion	Hemodialysis	eGFR <30 ml/min with/without HD
Group A	Levofloxacin[45]	750–1000 mg/day	kidney (87%), half-life 6–8 h 4[46]	Dialysis (30–50%), recommended administration after HD/on the day of HD[15]	750–1000 mg 3x/week
	Moxifloxacin[21]	400 mg/day	liver[41]	undialysis[15]	no dose adjustment required
	Bedaquilin[23]	400 mg/day the first 2 weeks, 200 mg/day next	predominant feces, a small part in the kidneys	undialysis[27]	no dose adjustment required
			long half-life		close monitoring in severe renal impairment
	Linezolid[23]	600 mg/day	liver[47]	minimal dialysis (20%) recommended administration after HD/in HD day[16]	no dose adjustment required
Group B	Klofazimin[21,23]	100 mg/day	predominant liver, a small portion of urine, sputum, sebum, sweat[48]	Undialysis[27]	no dose adjustment required
	Cycloserine[21]	10–15 mg/kg/day	renal predominant[49]	dialysis (56%), recommendations for administration after HD[27,50]	250 mg/day or 500mg/3x a week
Group C	Etambutol[20]	15–25 mg/kg/day	20% feces, 50% urine[49]	dialysis (2–40%), recommended administration after HD[50]	15–25 mg/kg/times, 3x a week
	Delamanid[21]	2 × 100 mg	not excreted in kidneys, minor metabolites in feces (26%) and urine (60%)[27]	no data[27]	No adjustments needed (not recommended due to limited data)
	Pyrazinamide[20]	20–30 mg/kg/day	Renal predominant[49]	Dialysis (45–50%), recommended administration after HD[15]	25–35 mg/kg/times, 3x a week
	Imipenem-silastatin[27]	500 mg/6 h or 1gr/8 h intravenous	Renal predominant	dialysis (25–32%), recommendations for administration after HD/on the day of HD[15]	250–500 mg/12 h
	Meropenem[27]	500 mg - 2 gr/8 h	Renal predominant	dialysis (51%) recommended administration after HD/in HD day[15]	1 g/12 h
	Amikacin/[21]	15–20 mg/kg/day	Renal predominant[23]	dialysis (95%) recommended administration after HD/in day HD[15]	12–15 mg/time, 2–3x a week
	Streptomycin[21]	12–18 mg/kg/day	Renal predominant[23]	dialysis, recommended administration after HD/in HD day[15]	12–15 mg/time, 2–3x a week
	Ethionamide[23]	15–20 mg/day in divided doses	Predominant liver	minimal dialysis recommended administration after HD[15]	No dose adjustment required
	P-Aminosalicylic acid[23]	8–12 g/day (2–3 divided doses)	Renal predominant	Dialysis, recommended administration after HD[15]	4gr/time, 2x a day (not recommended)

Abbreviations: HD, Hemodialysis.

2.4. *New regimen selection for drug-resistant tuberculosis in hemodialysis patients*

In May 2022, WHO issued guidance recommending the use of BpaLM regimen for 6–9 months for RR/MDR TB, and BpaL regimen for pre XDR/XDR TB patients.[10] The TB-PRACTECAL study compared BPaLM, BPaL regimen, and controls (str) for 24 weeks and found that the BPaLM regimen provided the best efficacy. It was also found that the administration of BpaL regimen without moxifloxacin still has good efficacy in patients with fluoroquinolone resistance and can still be used. The cure rate in the BpaLM group reached 89% compared to the control group of 52%. This regimen also has fewer side effects than the previous str regimen with the most side effects being myelosuppression and peripheral neuropathy. However, these common side effects can be treated.[29]

Recent studies in renal failure patients with or without RRT have not been widely conducted. This BPaL study did not include patients with more than 2x the normal upper limit (ULN) creatinine values. In this study, 5 patients with increased creatinine exceeding external debt were obtained.[30] Therefore, this regimen must be administered with close monitoring.

2.5. *Bedaquiline*

Bedaquiline is a drug that has an anti-tuberculosis mechanism. The drug was first discovered by Jhonson and Jhonson and was accepted as a tuberculosis drug by the FDA in 2012 and became a new anti-tuberculosis drug in 40 years.[31] Bdq became one of the main drugs in the new regimen by WHO in 2018, replacing injectable drugs in the short-term therapy of drug-resistant TB.[20]

Bdq has a mechanism associated with the inhibition of the synthesis of adenosine triphosphate (ATP) mycobacteria. Bdq was administered orally and studies that administered this drug at varying doses showed bacterial activity related to drug concentration and the main efficacy indicator is the area under the curve (AUC). The drug is distributed in plasma and binds to proteins 99%.[32] Bdq is widely distributed to tissues such as the lungs and spleen but concentrations in the brain are few. Bdq is mainly eliminated through feces. In clinical studies, it was found that on stool examination 24 h after consumption, there was a content of 75–85% Bdq with an unchanged form; in urine it was only found $\leq$0.001% of an oral dose of the drug 24 h after consumption. The half-life of the effectivity of this drug is 24 h after administration.[27]

Bdq studies are primarily conducted on patients without renal impairment. Creatinine clearance does not affect the pharmacokinetic parameters of Bdq in mild to moderate renal failure, thus, this drug tends to be safe. In severe renal failure, it is likely to affect the pharmacokinetics of this drug so it needs close monitoring. The drug mostly binds to plasma proteins, thereby the chances of elimination through HD are very small. Research on Bdq in patients with creatinine clearance ranging from 40 to 227 ml/min showed no significant difference.[33] There are no specific studies of Bbq administration in patients with end-stage renal failures, however several studies on mice found a decrease in AUC-24-h levels in mice with renal impairment. This is likely due to plasma protein disorders in renal impairment.[34]

2.6. *Pretomanide*

Pretomanide is the newest class of drugs in the treatment of TB. The FDA received the drug in 2019 and is already recommended by WHO for MDR, pre-XDR, and XDR TB patients in the latest regimen. Pretomanide belongs to the class of nitroimidazopyrans (NAP), which is a nitroimidazole derivative. Pretomanide is bactericidal and can work to inhibit protein and lipid biosynthesis, reduce the availability of mycolic keto acid and affect the pentose phosphate pathway, causing the accumulation of phosphate sugars that eventually interfere with bacterial replication. Pretomanide also acts on hypoxic bacteria that do not replicate utilizing bacterial respiratory toxicity through nitrite oxide.[35]

The drug is administered orally at a dosage of 100–200 mg/day. The drug binds to plasma proteins (95%) with a half-life of 16–24 h. These drugs are predominantly metabolized through various metabolite pathways, such as oxidative and reductive before being eliminated. The drug is excreted predominantly through urine (65%) and 26% of feces but only 1% is eliminated in its initial form.[27] Currently, there is not much data on the administration of this drug in patients with renal impairment but an increase in serum creatinine (0.2 mg/dl from the average normal person) was obtained, which was associated with the concentration of the drug without being followed by the decrease of the blood urea which decreased again after the drug was discontinued. This is likely due to the inhibition of creatinine secretion in the proximal tubules of the kidneys and does not represent an impaired renal function.[36] There are no data and recommendations for dose adjustment on pretomanide administration.[10]

2.7. *Linezolid*

Linezolid is the first synthetically created TB new drug from the oxazolidinones class that was first introduced in 1978. Linezolid is an antituberculosis drug that can disrupt protein synthesis by binding to rRNA (30S and 50S) and inhibiting initiation complexes during protein synthesis. Initially, linezolid was used as an antibiotic in some lung and skin infections but now it is also used for the treatment of MDR and XDR TB.[37]

Linezolid is well absorbed orally (100%) and is not affected by the presence of food. Linezolid binds to plasma proteins about 31% with a half-life of 3.4–7.4 h in normal people and 7–8 h in end-stage renal disease (ESRD) patients.[27] In renal impairment there are no dose adjustments because total excretion of linezolid is not associated with creatinine clearance. In studies on the pharmacokinetics of a single dose of linezolid, it was found that excretion levels did not change in several grades of renal function. However, several studies have shown a relationship between side effects of hematological toxicity such as anemia, thrombocytopenia, and leukopenia with renal impairment, the cause of which until now has not been known.[28] In some studies, there was also a relationship between renal impairment and the accumulation of linezolid metabolites characterized by an increase in C_{min} > 8 mg/L which can cause an increased risk of side effects mainly hematologic toxicity.[38] Therefore, it is necessary to consider dose reduction in patients with a renal impairment which is 300mg/12 h.[39]

On HD for 3–4 h, about 30–50% dose of linezolid is excreted. HD can partially remove linezolid metabolites, but there is still a possibility of linezolid accumulation in the ESRD. Because some drugs are excreted during HD, linezolid should be given after HD is carried out in order to maintain the effectiveness of the drug, and in patients with HD, it is better to monitor linezolid blood levels with therapeutic drug monitoring (TDM) to achieve the maximum dose.[39,40]

2.8. *Moxifloxacin*

Moxifloxacin is a drug that belongs to the fourth-generation fluoroquinolone group developed by Bayer Pharmaceuticals in 1990. This drug is one of the new drugs in conjunction with pretomanid, Bdq, and rifapentine which is studied as a tuberculosis drug, especially for MDR-TB. Moxifloxacin works as a bactericidal by binding to the enzyme topoisomerase II (DNA gyrase), which causes inhibition of replication, transcription, and healing of bacterial DNA.[41]

Moxifloxacin can kill bacteria even if there is no protein synthesis activity or a dormant state. Therefore, moxifloxacin

can prevent the incidence of recurrent infections. In a study comparing moxifloxacin and levofloxacin high-dose therapy in sensitive or fluoroquinolone-resistant TB patients, moxifloxacin was found to have better effectiveness so that it could be considered as a therapy in drug-resistant TB.[42]

Moxifloxacin is administered orally at a dose of 400 mg/day. The drug is absorbed with more than 90% bioavailability at oral administration. The drug is also well distributed in tissues. The concentration of this drug in epithelial tissue, lung tissue, alveolar macrophages, and bronchial mucosa exceeds the minimum inhibitory concentration (MIC) value against mycobacterium TB. Moxifloxacin also has a high concentration in the brain and cerebrospinal fluid so it can be used as a therapy in TB meningitis.[27]

Moxifloxacin is mainly metabolized and excreted through the liver about 51% through the conjugation of glucuronoside and sulfate. About 45% of the drug is excreted in its initial form through feces (25%) and urine (20%), while 38% is excreted through feces in the form of sulfate conjugates and 14% is excreted in the urine in the form of glucuronide conjugates.[43]

In renal impairment, it does not require dose adjustment to administer moxifloxacin. One study found that the value of Cmax was not influenced by the level of renal impairment, including patients undergoing HD and continuous ambulatory peritoneal dialysis (CAPD).[44] This is different from levofloxacin which is also included in the fluoroquinolone group and is also used in MDR-TB patients. In levofloxacin dose adjustment is required because most of it is excreted in the kidneys and the level of the drug is affected by the degree of renal impairment.[23] Therefore, it may be considered the use of moxifloxacin in patients with renal impairment.

3. Conclusion

The condition of hemodialysis can disrupt the pharmacokinetics of TB treatment, reducing the effectiveness of the drug, and increasing the risk of toxicity and side effects. Treatment of drug-resistant TB requires a longer duration of therapy and more drugs, which can further increase the risk of side effects. The latest TB regimen introduced by WHO in 2022 provides a shorter alternative treatment. The drugs used on this new regimen are mostly safe for patients with renal impairment due to their dominant metabolism in the liver. Special precaution should be taken with the administration of linezolid, due to increased hematology side effects in CKD patients, as well as moxifloxacin and bedaquiline, which can lengthen the QTC interval and increase the risk of arrhythmias in CKD patients with hemodialysis. There has not been much research on the combination of drug-resistant TB treatment in patients with CKD with or without HD. However, in patients with normal kidney function, no significant side effects or evidence of increased kidney damage was found. More research is needed to confirm these findings in the renal failure patient population.

Author contribution

All authors contributed equally to this work. JJ and PDS conceptualized the article, researched the literature, and drafted the first manuscript. BPR and ECS participated in literature research and helped to draft, edit and revise the manuscript. TFP also contributed to designing the manuscript concept and reviewed the final draft. All authors read and approved the final version of the manuscript.

Funding

None.

Conflict of interest

The authors have none to declare.

REFERENCES

1. Ragonnet R, Trauer JM, Geard N, Scott N, McBryde ES. Profiling Mycobacterium tuberculosis transmission and the resulting disease burden in the five highest tuberculosis burden countries. *BMC Med*. 2019 Nov 22;17(1).
2. World Health Organization. *Global Tuberculosis Report 2021 [Internet]*; 2021. Available from: http://apps.who.int/bookorders.
3. Alipanah N, Jarlsberg L, Miller C, et al. Adherence interventions and outcomes of tuberculosis treatment: a systematic review and meta-analysis of trials and observational studies. *PLoS Med*. 2018 Jul 1;15(7).
4. Ishigami J, Matsushita K. Clinical epidemiology of infectious disease among patients with chronic kidney disease. *Clinical and Experimental Nephrology*. 2019;23:437–447. Springer Tokyo.
5. Alemu A, Bitew ZW, Diriba G, et al. Tuberculosis incidence in patients with chronic kidney disease: a systematic review and meta-analysis. *International Journal of Infectious Diseases*. 2022;122:188–201. Elsevier B.V.
6. Ostermann M, Palchaudhuri P, Riding A, Begum P, Milburn HJ. Incidence of tuberculosis is high in chronic kidney disease patients in South East England and drug resistance common. *Ren Fail*. 2016 Feb 7;38(2):256–261.
7. Nguyen DT, Graviss EA. Development and validation of a prognostic score to predict tuberculosis mortality. *J Infect*. 2018 Oct 1;77(4):283–290.
8. Saito N, Yoshii Y, Kaneko Y, et al. Impact of renal function-based anti-tuberculosis drug dosage adjustment on efficacy and safety outcomes in pulmonary tuberculosis complicated with chronic kidney disease. *BMC Infect Dis*. 2019 May 2;19(1).
9. Migliori GB, Tiberi S. WHO drug-resistant TB guidelines 2022: what is new? *International Journal of Tuberculosis and Lung Disease. International Union Against Tuberculosis and Lung Disease*. 2022;26:590–591.
10. World Health Organization. *Rapid Communication: Key Changes to the Treatment of Drug-Resistant Tuberculosis [Internet]*. Geneva; 2022. Available from: https://www.who.int/about/licensing.
11. Daugirdas J, Blake P, Ing T. In: Goolsby J, Vandetty L, eds. *Handbook of Dialysis*. fifth ed. Wolters Kluwer; 2015.
12. Lea-Henry TN, Carland JE, Stocker SL, Sevastos J, Roberts DM. Clinical pharmacokinetics in kidney disease: fundamental principles. *Clin J Am Soc Nephrol*. 2018 Jul 6;13(7):1085–1095.
13. Miners JO, Yang X, Knights KM, Zhang L. The role of the kidney in drug elimination: transport, metabolism, and the impact of kidney disease on drug clearance. *Clin Pharmacol Ther*. 2017 Sep 1;102(3):436–449.
14. Gotta V, Dao K, Rodieux F, Buclin T, Livio F, Pfister M. Guidance to develop individual dose recommendations for

patients on chronic hemodialysis. *Expert Rev Clin Pharmacol*. 2017;10:737–752. Taylor and Francis Ltd.
15. Pistolesi V, Morabito S, Mario F di, Regolisti G, Cantarelli C, Fiaccadori E. A guide to understanding antimicrobial drug dosing in critically ill patients on renal replacement therapy. *Antimicrob Agents Chemother*. 2019;63(8).
16. Heintz BH, Matzke GR, Dager WE. Antimicrobial dosing concepts and recommendations for critically Ill adult patients receiving continuous renal replacement therapy or intermittent hemodialysis. *Pharmacotherapy*. 2009;29:562–577.
17. Liu X, Dai C. Advances in understanding and management of residual renal function in patients with chronic kidney disease. *Kidney Dis*. 2016;2(4):187–196.
18. Smyth B, Jones C, Saunders J. Prescribing for patients on dialysis. *Aust Prescr*. 2016 Feb 1;39(1):21–24.
19. Singh R, Dwivedi SP, Gaharwar US, Meena R, Rajamani P, Prasad T. Recent updates on drug resistance in Mycobacterium tuberculosis. *J Appl Microbiol*. 2020;128:1547–1567. Blackwell Publishing Ltd.
20. World Health Organization. *WHO Operational Handbook on Tuberculosis. Module 4: Treatment - Drug-Resistant Tuberculosis Treatment*. Geneva. 2020.
21. *Direktorat Jenderal Pencegahan dan Pengendalian Penyakit, Kementerian Kesehatan Republik Indonesia*. Petunjuk Teknis Penatalaksanaan Tuberkulosis Resistan Obat di Indonesia; 2020.
22. Hu X, Zhang M, Bai H, et al. Antituberculosis drug-induced adverse events in the liver, kidneys, and blood: clinical profiles and pharmacogenetic predictors. *Clin Pharmacol Ther*. 2018 Aug 1;104(2):326–334.
23. Diseases Branch C. Treatment of tuberculosis in renal disease. *Guideline Version 4.0 [Internet]*. 2021;4:6–10. Available from: https://www.health.qld.gov.au/clinical-.
24. Khan F, Ismail M, Khan Q, Ali Z. Moxifloxacin-induced QT interval prolongation and torsades de pointes: a narrative review. Taylor and Francis Ltd *Expet Opin Drug Saf*. 2018;17:1029–1039.
25. Cohen K, Maartens G. A safety evaluation of bedaquiline for the treatment of multi-drug resistant tuberculosis. *Expet Opin Drug Saf*. 2019 Oct 3;18(10):875–882.
26. Assimon MM, Wang L, Pun PH, Winkelmayer WC, Flythe JE. Use of qt prolonging medications by hemodialysis patients and individuals without end-stage kidney disease. *J Am Heart Assoc*. 2020 Jul 7;9(13).
27. Grayson ML, C S, C S, et al. In: Cosgrove S, Crowe S, Hope W, McCarthy J, Mills J, et al., eds. *Kucers' the Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic, and Antiviral Drugs*. seventh ed. Grayson ML: CRC Press; 2017.
28. Dai Y, Jiang S, Chen X, et al. Analysis of the risk factors of linezolid-related haematological toxicity in Chinese patients. *J Clin Pharm Therapeut*. 2021 Jun 1;46(3):807–813.
29. Berry C, du Cros P, Fielding K, et al. TB-PRACTECAL: study protocol for a randomised, controlled, open-label, phase II–III trial to evaluate the safety and efficacy of regimens containing bedaquiline and pretomanid for the treatment of adult patients with pulmonary multidrug-resistant tuberculosis. *Trials*. 2022 Dec 1;23(1).
30. Conradie F, Diacon AH, Ngubane N, et al. Treatment of highly drug-resistant pulmonary tuberculosis. *N Engl J Med*. 2020 Mar 5;382(10):893–902.
31. Mahajan R. Bedaquiline: first FDA-approved tuberculosis drug in 40 years. *Int J Appl Basic Med Res*. 2013;3(1):1.
32. van Heeswijk RPG, Dannemann B, Hoetelmans RMW. Bedaquiline: a review of human pharmacokinetics and drug-drug interactions. *J Antimicrob Chemother*. 2014;69(9):2310–2318.
33. McLeay SC, Vis P, van Heeswijk RPG, Green B. Population pharmacokinetics of bedaquiline (TMC207), a novel antituberculosis drug. *Antimicrob Agents Chemother*. 2014;58(9):5315–5324.
34. Gour A, Dogra A, Sharma S, Wazir P, Nandi U. Effect of disease state on the pharmacokinetics of bedaquiline in renal-impaired and diabetic rats. *ACS Omega*. 2021 Mar 16;6(10):6934–6941.
35. Stancil SL, Mirzayev F, Abdel-Rahman SM. Profiling pretomanid as a therapeutic option for tb infection: evidence to date. Dove Medical Press Ltd *Drug Design, Development and Therapy*. 2021;15:2815–2830.
36. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Assessment of the effects of the nitroimidazo-oxazine PA-824 on renal function in healthy subjects. *Antimicrob Agents Chemother*. 2009;53(9):3726–3733.
37. Hashemian SMR, Farhadi T, Ganjparvar M. Linezolid: a review of its properties, function, and use in critical care. Dove Medical Press Ltd. *Drug Design, Development and Therapy*. 2018;12:1759–1767
38. Fang J, Chen C, Wu Y, et al. Does the conventional dosage of linezolid necessitate therapeutic drug monitoring?—experience from a prospective observational study. *Ann Transl Med*. 2020 Apr;8(7):493, 493.
39. Ripamonti D, Cattaneo D, Sacco Hospital L, Antonio ID, Dai H, Copyright fpubh, et al. Expert Consensus Statement on Therapeutic Drug Monitoring and Individualization of Linezolid.
40. Rao GG, Konicki R, Cattaneo D, Alffenaar JW, Marriott DJE, Neely M. Therapeutic drug monitoring can improve linezolid dosing regimens in current clinical practice: a review of linezolid pharmacokinetics and pharmacodynamics. *Ther Drug Monit*. 2020 Feb 1;42(1):83–92.
41. Naidoo A, Naidoo K, McIlleron H, Essack S, Padayatchi N. A review of moxifloxacin for the treatment of drug-susceptible tuberculosis. *J Clin Pharmacol*. 2017;57:1369–1386. Blackwell Publishing Inc.
42. Maitre T, Petitjean G, Chauffour A, et al. Are moxifloxacin and levofloxacin equally effective to treat XDR tuberculosis? *J Antimicrob Chemother*. 2017 Aug 1;72(8):2326–2333.
43. Stass H, Kubitza D. Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man. *J Antimicrob Chemother*. 1999;43:83–90.
44. Stass H, Kubitza D, Halabi A, Delesen H. Pharmacokinetics of moxifloxacin, a novel 8-methoxy-quinolone, in patients with renal dysfunction. *Br J Clin Pharmacol*. 2002;53(3):232–237.
45. Perhimpunan Dokter Paru Indonesia (PDPI). Jakarta. In: Isbaniah F, Burhan E, Sinaga B, Yanifitri D, Handayani D, eds. *Pedoman Diagnosis dan Peatalaksanaan di Indonesia. Edisi Revisi 3*. 2021.
46. Podder V, Sadiq NM. *Levofloxacin*. 2022.
47. Fermeli DD, Marantos TD, Liarakos ALD, Panayiotakopoulos GD, Dedes VK, Panoutsopoulos GI. Linezolid: a promising agent for the treatment of multiple and extensively drug-resistant tuberculosis. *Folia Med (Plovdiv)*. 2020 Sep 30;62(3):444–452.
48. McGuffin SA, Pottinger PS, Harnisch JP. Clofazimine in nontuberculous mycobacterial infections: a growing niche. *Open Forum Infect Dis*. 2017 Jul 1;4(3).
49. Marshall G, Raveendran RR. *Basics of Pharmacology and Toxicology Volume 1: General and Molecular Pharmacology: Principles of Drug Action Introduction to Basics of Pharmacology and Toxicology*. 2019.
50. Pien FD, Younoszai BG, Pien BC. Mycobacterial infections in patients with chronic renal disease. *Infect Dis Clin North Am*. 2001;15(3):851–871.

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/indian-journal-of-tuberculosis/

Review article

From nature's bounty to drug discovery: Leveraging phytochemicals and molecular approaches to combat multi-drug-resistant (MDR) tuberculosis

Sarveswara Mounik Nalam [a], *Pavan Kumar Chintamaneni* [b], *Rashmi Saxena Pal* [a], *M.V.N.L. Chaitanya* [a,*], *Sachin Kumar Singh* [a], *P. Saranya* [c], *Smriti Arora* [d], *Sarika Sharma* [e], *Pratibha Pandey* [f], *Avijit Mazumder* [g], *Ravindra Babu* [a], *Patrick Amoateng* [h], *Amandeep Singh* [i]

[a] *School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, 144402, India*
[b] *Department of Pharmaceutics, GITAM School of Pharmacy, Gitam-Hyderabad Campus, Hyderabad, 502329, India*
[c] *Department of Pharmacy Practice, Faculty of Pharmacy, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research, Chromepet, Chennai, 600044, India*
[d] *Department of Allied Health Sciences, University of Petroleum & Energy Studies (UPES), Bidholi, Dehradun, 248007, India*
[e] *Department of Sponsored Research, Division of Research & Development, Lovely Professional University, Phagwara, 144402, India*
[f] *Noida Institute of Engineering & Technology, Gautam Buddh Nagar, 19, Knowledge Park-II, Institutional Area, Greater Noida, 201306, India*
[g] *Niet Pharmacy Institute C Block, Noida Institute of Engineering & Technology, Gautam Buddh Nagar, 19, Knowledge Park-II, Institutional Area, Greater Noida, 201306, India*
[h] *Pharmacology & Toxicology, School of Pharmacy, University of Ghana, Legon, Accra, India*
[i] *Khalsa College of Pharmacy, H.No 21a, Lane No 4, Chheharta, Amritsar, Punjab, 143002, India*

ARTICLE INFO

Article history:
Received 25 June 2023
Received in revised form
7 August 2023
Accepted 10 August 2023
Available online 19 August 2023

ABSTRACT

A large number of people annually lose their lives to tuberculosis (TB), which is an age-old disease caused by the *Mycobacterium tuberculosis*. The global spread of TB is a concern for all regions. The south-east Asian region recorded 46% of all new TB cases in 2021, followed by the African and western Pacific regions with 23% and 18%, respectively. Researchers are always searching at natural substances for potential alternative therapeutics to tackle the worrisome growth in multi-drug-resistant (MDR) tuberculosis due to the high costs associated with developing new treatments and unfavourable side effects of currently used

Abbreviations: TB, Tuberculosis; HIV, Human immunodeficiency virus; AIDS, Acquired immunodeficiency syndrome; WHO, World health organization; NRHM, National rural health mission; NTCP, National tobacco control program; IMA, Indian medical association; UTI, Urinary tract infection; EPTB, Extra pulmonary tuberculosis; MDR, Multi drug resistant; *M. tuberculosis*, *Mycobacterium tuberculosis*; EPTB, Extrapulmonary TB; Bdq, Bedaquiline; GTB, Genitourinary TB; LR, Linezolide resistance; DR, Delamanid resistance; CR, Clofazimine resistance; PR, Pretomanid resistance; NFB, Nuclear factor-kappa B; IFN-γ, interferon-gamma (IFN-γ); MAPKs, MTB-infected macrophages; TLR2, Toll-like receptor 2.

* *Corresponding author.*
E-mail address: chaitanya.phyto@gmail.com (M.V.N.L. Chaitanya).

https://doi.org/10.1016/j.ijtb.2023.08.007

Keywords:
Mycobacterium tuberculosis
Natural resources
Nanoparticles
Tuberculosis
Ayurveda

synthetic pharmaceuticals. Phytochemicals show promising results as a future health aid due to their multi-targeting ability on pathogen cells. In the search for new drug leads, the Ayurvedic and Siddha medical systems have made an extensive use of ethnomedicinal tools, including the use of plants like Amalaki (*Emblica officinalis* Gaertn.), Guduchi (*Tinospora cordifolia* willd.), Sariva (*Hemidesmus indicus* R.Br.), Kustha (*Saussurea lappa* Falc.), turmeric (*Curcuma longa* Mal.) and Green tea (*Camellia sinensis* Linn.). These sources are high in flavonoids, polyphenols, tannins and catechins, has been shown to reduce the risk of TB. In this overview, we look at how natural sources like plants, algae and mushrooms have helped researchers to find new drug leads, and how to back these natural sources through mapping the molecular approaches and other approaches has helped them to defeat MDR.

1. Background

A total of 63 articles used the tools such as PubMed, Embase, and Mendeley in order to conduct research on the discovery of natural sources that may be useful for treating tuberculosis in accordance with the table of contents. As a result, these tools have been extensively used by us to fill the gaps in the existing information about tuberculosis and plants. A variety of Scopus journals were searched during the process of gathering the literature and filling the research gaps, which were collected from a variety of sources in order to fill the research gaps using the keywords such as global statistics on Tuberculosis, antitubercular drugs, phytochemicals, secondary metabolites, traditional medicine in treating tuberculosis, Ayurveda, molecular mechanisms.

2. Introduction

Tuberculosis (TB) is an age-old disease caused by the "*Mycobacterium tuberculosis* (MT)", a member of the Mycobacteriaceae family and is endemic to Africa. It primarily affects the lungs but can also affect the lymph nodes, intestines, bones, liver, spleen, kidneys, brain, and spine. An infected person can transfer TB to others through sneezing or coughing. TB was categorized in two ways, viz., active TB which promotes the disease's spread from one host to another and latent tuberculosis cells are less harmful than active TB cells because they don't multiply rapidly or damage the body. However, if the disease isn't treated in its early, non-contagious stages, it can spread from person to person.[1] About one-third of the global population has a latent case of TB. Tubercles, which are small nodular lesions in the lungs and other tissues, are caused by *Mycobacterium tuberculosis* and TB refers to the process and condition brought on by tubercules. The incidence of TB is not recent, evidence of its existence in ancient Egypt and Africa 150 million years ago. However, tuberculosis, initially recognized as a group of mycobacteria in the early 1820s, received its official name "tuberculosis" in 1839, by the efforts of J. L. Schoenlein. Later, in 1882, Robert Koch successfully identified *Mycobacterium tuberculosis* (MT) as the specific bacterium responsible for causing tuberculosis, for which he earned the Nobel Prize in Medicine in 1905.

Tuberculosis (TB) can affect different parts of the body, but the most common site of infection is the lungs, however, infection in extrapulmonary sites was also reported. It's important to distinguish between pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis, (EPTB) Pulmonary TB is the most common form of TB and is usually characterized by a persistent cough, sometimes accompanied by bloody sputum, chest pain, shortness of breath, fatigue, weight loss, and fever. These symptoms may develop gradually over several weeks or months, and the severity can vary depending on the extent of the lung involvement. In some cases, TB may be asymptomatic, particularly in immunocompromised individuals, such as populations infected with HIV. Only about 15% of TB cases are considered extrapulmonary TB, and there is a dearth of data on this subtype. Extrapulmonary TB refers to TB that affects parts of the body other than the lungs. The most common sites of extrapulmonary TB (EPTB) include the lymph nodes, bones, joints, and the central nervous system. In 2021, TB diagnoses reached 10.6 million, a 4.5% increase from 2020. Approximately 82% of non-HIV TB deaths occurred in Africa and Southeast Asia. In India, the projected TB rate for 2021 is 210 cases per 100,000 people, up from the 2015 estimate of 105 cases per 100,000 people. Despite an 18% decline in TB deaths compared to previous years, there was a 3% global rise in multi-drug-resistant TB (MDR-TB), with 450,000 cases identified as rifampicin-resistant tuberculosis (RR- TB).[2]

3. Multi-drug resistant tuberculosis (MDR-TB)

TB that is resistant to antibiotics is a major public health problem in many parts of the world and the prevalence of MDR-TB, in which the causative agent, *Mycobacterium tuberculosis*, is resistant to multiple antituberculosis medications like rifampicin and isoniazid[3] have increased dramatically over the past decade. It was clearly evident from the reported literature that TB is becoming resistance to newer approval drugs like bedaquiline (Bdq),[4] linezolid,[5] delamanid, clofazimine,[6] and pretomanid.[7] However, the MDR-TB had not shown any resistance to some of the new drug like sutezolid.[8] The discovery of new drug leads to treat MDR-TB relies on an understanding of the mechanisms by which TB develops resistance to these new treatments. To understand various

MDR pathways by the *M. tuberculosis* is explained below based on various reported evidences Fig. 1.

4. MDR-TB mechanisms

4.1. *Bedaquiline resistance (BDQR)*

Bedaquiline, delamanid, and pretomanid are the three novel medicines licenced during the previous decade, have fundamentally changed the way TB is treated.

Bedaquiline (BD), was first recommended for treatment of multidrug-resistant TB in, Taiwan in 2014. However, with excessive use of BD, cases of BDQR began to raise, accordingly a retrospective population-based study carried out by Wu et al in 2021. In this retrospective population-based investigation, 898 instances of rifampicin- or multidrug-resistant tuberculosis (MDR-TB) from 2008 to 2019 were included. From these instances, 65 isolates were chosen at random, 28 of which had BDQ MICs of less than 0.25 g/mL, and the remaining 28 were separated into study and control groups. Using the MGIT960 system for BDQ drug susceptibility testing (DST), the atpE, Rv0678, and pepQ genes were sequenced using Sanger technology. 38.9% of the isolates with MGIT-BDQ resistance and 61.1% of the isolates with BDQ MIC = 0.25 g/mL were MGIT-BDQ susceptible. In order to resolve differences in DST technique. Out of the 93 isolates, 22 carried Rv0678 mutations and were MGIT-BDQ resistant. In all of the remaining MGIT-BDQ-resistant isolates, there were seven new mutations in the Rv0678 gene, constituting 100% of the mutations. While 22.7% of isolates with borderline MGIT-BDQ resistance lacked changes in the examined genes, these isolates nonetheless exhibited resistance to the drug. In order to rule out BDQ resistance in isolates with phenotypic MGIT-BDQ borderline resistance, we advise examining GU differences or performing genotypic investigations. Notably, BDQ-containing regimens were effective in treating patients with BDQ-resistant isolates, regardless of Rv0678 mutations. Finally, 3.1% of drug-resistant TB cases without a history of exposure to BDQ exhibited BDQ resistance based on MIC0.25g/mL. Although its mutations had no effect on the effectiveness of treatment, Rv0678 was an unreliable diagnostic for BDQ resistance. *M. tuberculosis*, however, has begun expressing genetic changes against BdQ, frightening the global community and necessitating new medications to treat MDR-TB worldwide, which can be achieved only through drug development studies based on alternative medicine.[9] The BdQ and CFt resistance in RVO6t8 mutants was represented in Fig. 2.

(reproduced under Creative Commons Attribution License from **ref: Acquired Resistance of *Mycobacterium tuberculosis* to Bedaquiline**).

4.2. *Linezolid resistance (LR)*

The MDR-TB pathogen was also expressed the resistance to Linezolid. However, little is known about the mutations that confer resistance to linezolid, despite the fact that it is rapidly becoming an important antibiotic for treating MDR/XDR TB. As per the study carried out by Pi et al, in 2019, an *in-vitro* parallel selections of linezolid-resistant isolates to examine the mutations associated with this MDR at the genome level were used. In the current study *M. tuberculosis* (H37Rv) was grown in two different places, and on 7H11 agar plates with 2.5 mg/L linezolid, spontaneous mutations were chosen. It was observed that colonies that were resistant to linezolid were tested further by growing them on a second plate of linezolid. Then, whole-genome sequencing (WGS) was used to find mutations that cause LR. Initially, 181 colonies were found to be resistant to linezolid, but 154 of those were later confirmed. In 88.3% (136/154) of these isolates, whole genome sequencing revealed that T460C mutations in rplC resulted in C154R substitutions.

All but 18 of the isolates had single mutations in the rrl gene, with 7.8% (12/154) having the G2814T mutation and 3.9% (6/154) having the G2270T mutation. Based on these findings,

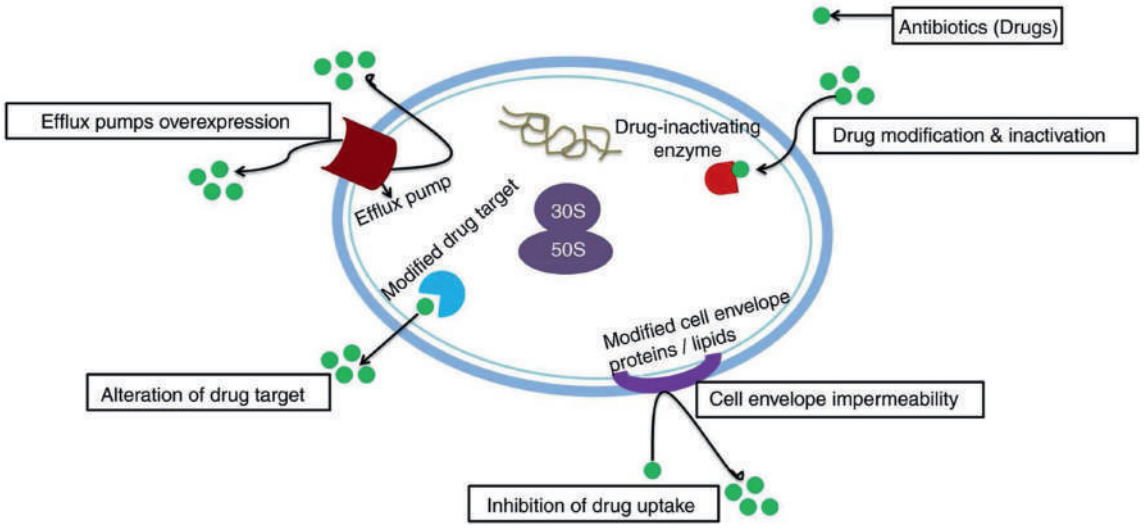


Fig. 1 – Schematic diagram showing the drug resistance mechanisms in *Mycobacterium tuberculosis*. Ref (Recent updates on drug resistance in *Mycobacterium tuberculosis*).

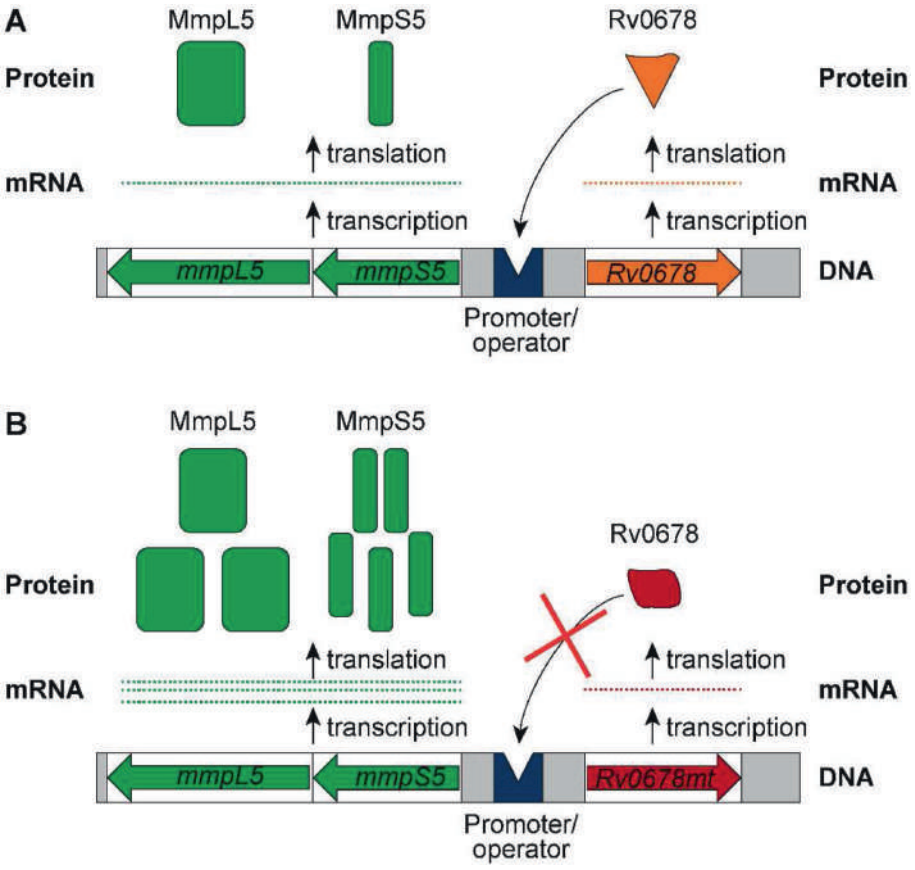


Fig. 2 – Mechanism of BDQ and CFZ resistance in Rv0678 mutants.

molecular testing for linezolid resistance should focus primarily on the rrl and rplC genes.[10]

4.3. *Delamanid resistance (DR)*

Researchers have found evidence that *M. tuberculosis* is resistant to the drug Delamanid. In 2020, scientists Reichmuth et al found that a genetic polymorphism accounts for the DR in *M. tuberculosis*. Delamanid resistance (DR) can be caused by mutations in ddn, fgd1, fbiA, fbiB, fbiC, and fbiD, all of which are involved in the F420 signalling pathway of the *M. tuberculosis* complex. It was also observed that one strain isolated from a patient who had never taken a Delamanid before was found to have a DR due to a naturally occurring polymorphism called Tyr29del (ddn).[11] The DR of the MTB was represented in Fig. 3.

4.4. *Clofazimine resistance (CR)*

The mechanism of clofazimine resistance in *Mycobacterium abscessus* remains unknown, despite the fact that clofazimine is an efficient medication active against *M. abscessus*. The first CR resistance mycobacterium strain and case was observed by Chen et al in 2018. In order to learn more about the genetics behind clofazimine resistance in *M. abscessus*, they sequenced the whole genomes of 29 clofazimine-resistant *M. abscessus* mutants. Resistance to clofazimine was shown to be most frequently caused by changes in three genes: MAB 2299c (encoding a potential transcriptional regulatory protein), MAB 1483 and MAB 0540. Resistance to clofazimine was also linked to mutations in MAB 0416c, MAB 4099c, MAB 2613, MAB 0409, and MAB 1426, albeit at a lower frequency. A total of 13 mutants shared two identical mutations, in MAB 4605c and MAB 4323, that are likely to be polymorphisms unrelated to CR. The study found that clofazimine resistance in *M. abscessus* is mostly caused by mutations in the genes MAB 2299c, MAB 1483, and MAB 0540. The observed mutations may play a role in *M. abscessus*'s resistance to clofazimine, but the researchers claimed that there is need to be investigated further in future investigations. The current research have implications for identifying clofazimine resistance early on and for understanding the mechanisms of CR.[6–12]

4.5. *Pretomanid resistance (PR)*

Pretomanid, previously known as PA-824 is a novel oral bicyclic nitroimidazooxazine. Treatment of pulmonary XDR-TB and treatment of intolerant or nonresponsive (TI/NR)/MDR-TB with pretomanid (200 mg), bedaquiline (400 mg/200 mg), and linezolid (1200 mg) (BPaL) was authorized by the FDA on August 14, 2019. Rifat et al. 2020 were the first researchers to

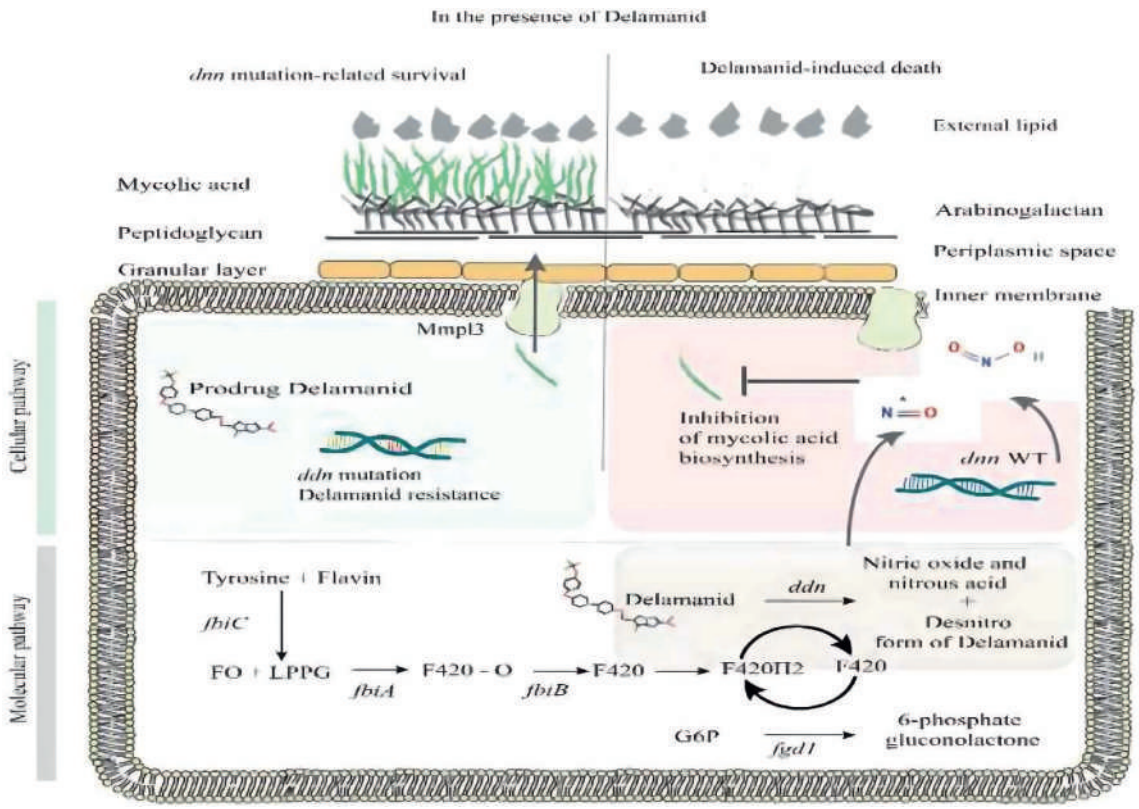


Fig. 3 – Mechanism of delamanid resistance. (reproduced under Creative Commons Attribution License (CC BY). ref: Mechanism of Action, Resistance, Synergism, and Clinical Implications of Delamanid Against Multidrug-Resistant *Mycobacterium tuberculosis*).

identify and report the PR resistance genes from MDR-TB-infected mouse models. Using whole-genome sequencing, researchers found 99 unique mutations in 161 resistant isolates from 47 mice, with 91% of those mutations occurring in 1 of 5 genes previously associated with nitroimidazole activation and resistance: fbiC (56%), fbiA (15%), ddn (12%), fgd (4%), and fbiB (4%).[7]

The rising prevalence of MDR-TB and the side effects of first- and second-line antitubercular drugs, evidence of promising new drug candidates derived from natural sources, have rekindled interest in novel antitubercular leads from natural sources.

Indeed, the discovery of new chemical molecules against active and latent tuberculosis (TB) from natural products is a formidable challenge that requires an interdisciplinary approach. Scientists working in this field face several obstacles, such as drug resistance and the complex nature of the TB-causing bacterium, *Mycobacterium tuberculosis*. Plants, with their rich chemotype diversity, may provide a useful new resource for anti-tuberculosis medications.

Despite India's estimated 17,500 higher plant species, only roughly 365 have been investigated for antimycobacterial activity.[13] Here, we review set the potential of phytochemicals with antitubercular activity, and the biological materials from which they are derived in order to identify potential new antitubercular drug leads, which are urgently needed for treating MDR-TB.

5. Therapeutic potential of Indian traditional herbs for tuberculosis treatment

5.1. Curcuma longa *Linn*

In Ayurveda, turmeric known as 'haridra' (a Sanskrit term), and is used to treat a wide variety of conditions, including: asthma, bronchial hyperactivity, allergies, liver diseases, anorexia, rheumatism, diabetic wounds, a persistent cough, and a stuffy or runny nose. Traditional Chinese medicine prescribes it for indigestion.[14] The primary natural polyphenol in the rhizome of *C. longa* (turmeric) and other Curcuma spp. is curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), also known as diferuloylmethane.[15] Curcumin has recently been linked to an improvement in MDR-TB. As per the research carried out by Bai et al. 2016, curcumin was found to be a potent inducer of apoptosis, an effector mechanism employed by macrophages to destroy intracellular *M. tuberculosis*. and it was found to improve MTB clearance in both human primary alveolar macrophages and THP-1 monocytes. These anti-MTB cellular functions were mediated by curcumin and also by preventing nuclear factor-kappa B (NFB) activation.[16] As per the research carried out by Marini et al. 2018, on the *M. abscessus* strain, curcumin shod a synergistic activity against the strain 29904 along with broad-spectrum antibiotics like linezolid, ciprofloxacin, amikacin,

and clarithromycin, in which the minimum inhibitory concentration observed was 128 mg/mL. At 1/8 MIC, curcumin significantly reduced motility, and at 4 MIC, it was completely inhibited microbial biofilms that had been matured for 4 and 8 days.[17]

Curcumin reduces macrophage apoptosis caused by P19 by modulating the c-Jun N-terminal kinase (JNK) pathway. Blocking the p38 MAPK signalling pathway in human macrophages cell line WBC264-9C by combining 20 and 40 mol/L concentrations of curcumin with the *M. tuberculosis* and it was demonstrated that curcumin reduced the inflammatory responses and apoptosis caused by curcumin significantly reduced P19-induced growth inhibition ($p \leq 0.01$), as measured by significantly decreased expression of the cytokines IL-1, IL-6, TNF, the signal transduction and transcription activator 3 (STAT3), and apoptotic proteins P53, Bax, Bcl2, and phospho-p38 MAPK in the presence and absence of the p38 MAPK antagonist. Curcumin's antitubercular action via regulation of the host immune response is unknown, and more research is required to determine its protective effect of curcumin in MTB-infected macrophages.[14,15] Although preliminary evidence suggests that curcumin may be effective against MDR-TB, more in-depth clinical studies are needed to confirm the efficacy of curcumin.

5.2. Bauhinia vahlii *Wight*

The Bauhinia genus is highly regarded in Ayurvedic medicine for its effectiveness in combating tuberculous and lymphadenitis. In Sanskrit, it is known as 'kanchanara' and has the capability to balance kapha and pitta doshas. In Ayurveda, flowers, and roots from the Bauhinia genus are highly regarded for their ability to heal scrofula, tuberculosis lymphadenitis, worm infestation, and wounds. This genus exhibits diverse biological activities, such as antimycobacterial activity, due in large part to the presence of numerous bioactive compounds like triterpenoid flavonoids, saponins, and phenanthroquinones.[18] In addition, drug leads like kaempferol, ombuin, and quercetin were isolated from the bark, supporting the antimycobacterial activity of *Bauhinia vahlii*. Ombuin's IC_{50} values against latent mycobacterium strains ranged from 2.85 ± 0.14 to 7.21 ± 1.09 nM, making it the flavonoid with the most potent antimicrobial activity.[19] Clinical evidences and molecular mechanisms of Bauhinia sp., against MDR-TB were not reported till today. However, there is a significant need to verify how ombuin can convert as drug lead, and to fill this void using cutting-edge drug discovery methods.

5.3. Acalypha indica *Linn*

Acalypha indica, sometimes referred to as 'haritamanjari' and a weed that grows all throughout India, which is listed in the Ayurvedic Pharmacopoeia. This herb is used in Ayurveda to cure a number of conditions, including toothaches, coughs, asthma, arthritis, and constipation. The major chemical constituents are alkaloids like acalyphine (alkaloid). Acalyphamide (sterol), kaempferol (flavanol) have been reported from this plant.[20] Research carried out by Bernaitis et al. 2013, It was observed that the ethanolic extracts of *A. indica* exhibited significant mycotoxic activity with proportion of inhibition of 75% against the MDR-TB strains of *M. tuberculosis* (H37Rv) and *Mycobacterium fortuitum* (TMC1529). However, the reported research is restricted only to *in vitro* studies on the resistant strains of MDR-TB. This research had laid down an innovative scope for the in-depth research on *A.indica* to discover new drug leads and their clinical validation.[21] This work has established a novel framework for future in-depth studies of *A.indica*, with the goal of identifying potential new therapeutic leads and validating these leads in clinical trials.

5.4. Abies spectabilis *(D.Don) Mirb*

Abies spectabilis (Talisa) is a plant used in Ayurvedic medicine for the treatment of respiratory disorders (Shwashara) and skin disorders (Kasahara); its common Sanskrit name is 'Patradyam' (in treating cough and cold). It consists of polyphenols, including terpenoids, flavonoids, and lignans like 7 α-methoxy-dehydroabietic acid[22] etc. But until now, there have been no attempts to scientifically prove the antituberculosis activity of Abies sp., and the only evidences available are from ayurvedic literature. Therefore, there are numerous openings for today's scientists to find new drug leads to combat MDR-TB from *A. spectabilis*.

5.5. Adhatoda Vasica *Nees*

Adhatoda Vasica Nees. is a plant of ethnomedicinal importance that belongs to the Acanthaceae family and has traditionally been used in the Southeast tropical zone to colds, whooping cough, and bronchial infections, and it has remarkable pharmacological properties.[23] The plant was used in traditional Indian medicine at least 2000 years ago, according to archaeological findings[24] and it was observed that the *Adathodai vasica* extracts, fractions, and their biomolecules have shown a significant antimycobacterial activity. Hydroalcoholic extracts of *A. vasica* were fractionated using bioassay guidance into hexane, ethyl acetate, and methanol; the results showed that the hexane fraction significantly reduced colony-forming units at 100 μg/mL, prompting further investigation into the plant's phytochemical nature. Vasicine acetate and 2-acetyl benzylamine, out of many phytochemical metabolites, were isolated from the hexane fraction and showed significant antitubercular activity with IC_{50} values of 200 and 50 μg/mL, respectively.[25] In BCG-infected THP1 macrophages, it had little anti-proliferative action and no phagolysosome fusion. Vasicine, on the other hand, synergized with INH for phagolysosome fusion. From the above findings we can say that *Adhatoda* contains bioactive chemicals that are responsible for anti-tubercular action, which should be investigated further for anti-TB medication development.[26] But these drug leads still need more in-depth research to demonstrate their clinical safety and efficacy.

5.6. Glycyrrhiza glabra *var. glandulifera*

Glycyrrhiza glabra (also known as licorice) is a member of the Fabaceae family, is used to treat inflammations, eye diseases, and throat infections, and is known as 'yastimadhu' in

Ayurveda because it balances vata and kapha doshas. The phytochemical and *in-vitro* studies have been carried out on *G. glabra* and it was found to be that this plant is a storehouse of important isoflavone metabolites like glabridin which was proven to be an important antitubercular drug lead. It was significantly active against pathogenic *M. tuberculosis* (H37Rv and H37Ra strains) with IC_{50} values of 29.16 μg/mL.[27] Hispaglabridin- B is another active constituents of *G. glabra*, which are responsible for antitubercular activity. The other phenolic compounds found antitubercular phenolic compounds from *G. glabra* and *G. inflate* were licoisoflavone and licochalcone.The antitubercular activity of glabridin was found to be 20 times more than that of crude extract in this investigation (ethanolic extract). BACTEC test revealed that root of ethanolic extract has antimycobacterial activity against Mycobacterium TB H37Ra and H37Rv strains at 500 g/mL. The MIC of the test substances was determined using the GI (growth index) value.[28] Glabridin has two free phenolic hydroxyls at 1,3-positions, which may be an important in generating action. Hispaglabridin' s inactivity might be attributed to one of the hydroxyl groups being shielded by an isoprenyl group as a benzopyrene ring. With the identification of glabridin as a powerful lead molecule for antimycobacterial action. It was observed that the results lend credence to the use of *G.glabra* in traditional medicine for the treatment of coughs and other chest complaints. It's possible that studying its structure—activity relationship (SAR) will help with the development of a superior drug in the not-too-distant future.[29]

5.7. Tinospora cardifolia *Wild*

India has long utilised *Tinospora cordifolia*, commonly known as Guduchi or "Amrita" in Ayurvedic medicine, to cure a variety of illnesses, including TB. *T.cordifolia* has been demonstrated in studies to strengthen the immune system and prevent the growth of *M. tuberculosis*, the bacteria that causes TB, suggesting that it may act as adjuvant therapy for the disease by maintaining the balance in three doshas vata, pitta, and Kapha. Several studies suggest that *T.cordifolia's* metabolites may have a number of beneficial effects that could be useful in the fight against TB.[30,31] Further, an *in vitro and ex vivo* macrophage model of MTB infection, G1-4A (a polysaccharide obtained from *T. cordifolia*) inhibited the survival of both drug-sensitive and multi-drug resistant (MDR-TB) strains by inducing nitric oxide (NO) in a TLR4-MyD88 dependent manner, along with inducing pro-inflammatory cytokines and surface expression of MHC-II and CD-86. In MTB-infected BALB/c mice, it induces a Th1 immune response and reduces lung bacterial load.[32] Cell-mediated immunity, and more specifically the Th1 response, is crucial for the effective management and eventual eradication of tuberculosis. Inducing bacterial killing in infected macrophages via the release of tumour necrosis factor TNF-α and nitric oxide, IFN- and other Th1 cytokines play a crucial role in the treatment of MTB infection. After *in vitro* PPD restimulation, splenocytes treated with G1-4A showed evidence of Th1 polarised cells, as measured by an increase in IFN- and a decrease in IL-4. IFN-α activates macrophages, and these cells then kill MTB via a NO-dependent pathways.[33]

5.8. Alpinia galanga (L.) *Wild*

Galangal, also called Thai ginger, is the root of the *Alpinia galanga* plant and has long been used in Southeast Asian medicine as an anti-inflammatory, anti-tubercular agent, and antioxidant, anti-inflammatory, and antibacterial properties have all been attributed to components of *A. galanga*.[34] However, there is less evidence supporting this antitubercular activity. However, the phytochemical *galangin* isolated from this plant has shown promise in preventing the spread of tuberculin-causing bacteria in some studies. In another study found that *A. galanga* rhizome extract containing a flavonoid had a modest anti-tuberculosis effect.[35] These studies, however, should be interpreted with caution because they were conducted on isolated chemicals or extracts rather than the whole plant. Thus, *A. galanga* needs more research to prove its potential as an antibacterial agent and its efficacy in treating TB.[36]

5.9. Hemidesmus indicus *L. Br*

H. Indicus is a perennial plant native to India and Sri Lanka, *Hemidesmus indicus* can also be found in those countries. Ayurvedic practitioners use it to treat a variety of conditions, such as fever, skin issues, and urinary tract infections; it also promotes overall body rejuvenation and supports the maintenance of vata, pitta, and kapha. It is also used to cleanse the blood and improve overall health and wellness. The herb has long been utilized in Ayurvedic medicine to treat respiratory illnesses such as tuberculosis. Plant metabolite compounds like anhydrous-sarsasapogenin and sarsaparilloside have anti-inflammatory and immunomodulatory properties that could be useful in the fight against tuberculosis.[37,38] *In-vitro* and *in-vivo* studies have shown that *H.indicus* may have a role in TB treatment. Since it has been shown to limit the development of *M. tuberculosis* and to have an immunological modulatory impact. More study is needed to fully understand the possible therapeutic advantages of *H.indicus* in treating TB, and it should never be used as the only therapy for TB.

5.10. Allium cepa *Linn*

In complementary and alternative medicine, the onion, or *Allium cepa*, is valued for its antioxidant and anti-inflammatory effects. Because of the high levels of vitamin C and fibre in both their raw and cooked forms, they are beneficial to our health in both forms. Traditional medicine practitioners have used *A. cepa* to treat a variety of conditions, including tuberculosis (TB). Numerous phytochemicals such as quercetin, rutin, allicin, and myricetin in *A.cepa* have been shown to have antibacterial properties and to inhibit the growth of tuberculosis-causing bacteria. It is believed the flavonoid quercetin in onions, exerts its antimicrobial properties primarily by inhibiting bacterial cell growth and causing membrane breakdown in bacterial cells.[39] It was also reported that Quercetin may inhibit the activity of growth-promoting enzymes in bacteria so that they can't multiply. As per the literature evidence, the allicin. having antibacterial effects. Scientific studies suggest that allicin can inhibit *M. tuberculosis* development by causing membrane

damage and ultimately killing the bacteria.[40] According to a possible structure–activity relationship, the presence of hydroxyl the 3, 4 positions (luteolin, quercetin, and myricetin) was required for M. TB inhibition. Rutin (quercetin-3-O-rutinoside) and quercetin (aglycone) have comparable physical and chemical properties; however, quercetin inhibits *M. tuberculosis* significantly more than rutin. This could be due to the compounds' rigid structure and binding specificity to mycobacterium.[41] However, more research is needed to determine the safety and efficacy of using *A.cepa* as a treatment for MDR-TB, as studies on the use of *A. Cepa* to treat MDR-TB are still in their early stages.

5.11. Pueraria tuberosa *Robx*

The medicinal potential of the plant *Pueraria tuberosa* has been investigated for its use in tuberculosis treatment (TB). Puerarin is one example of a phytochemical that has been shown in multiple studies to have anti-mycobacterial activity against *M. tuberculosis*. It can also hinder the virulence of *M. tuberculosis* by reducing the production of biofilm. Yet, more research is needed to ascertain *P. tuberosa*'s efficacy as an anti-TB medication in humans and to understand the mechanisms by which it acts. To date, there has been no clinical research on or approval of *P. tuberosa* for the treatment of tuberculosis till today. *P. tuberosa*'s molecular approach against MDR-TB is not fully known. Certain chemicals identified in *P. tuberosa*, such as puerarin and daidzin, have been demonstrated in studies to have antibiotic action against the microbe that causes tuberculosis.[42] Furthermore, some studies suggest that *P. tuberosa* has the potential to be used as an adjuvant therapy to boost the efficacy of standard TB therapies. More study, however, is required to completely understand the mechanistic approach of *P. tuberosa* for MDR-TB, as well as to evaluate its safety and efficacy.

5.12. Lentinula edodes *Berk*

Shiitake mushrooms (*Lentinula edodes*) are an edible fungus native to East Asia that is widely used in Asian cuisine. Shiitake mushrooms have been used in traditional medicine for ages in addition to their culinary use. It includes chemicals that have anti-inflammatory, antiviral, and immune-boosting activities. It had long been used in Chinese and Japanese medicine to cure a variety of diseases, including tuberculosis (TB). Shiitake mushrooms' active components, such as polysaccharides and beta-glucans, are thought to have an immunomodulatory quality that can help enhance the immune system and combat illnesses like TB. According to certain research, shiitake mushroom extract can increase the efficiency of traditional TB treatment and shorten the length of treatment. For example, shiitake mushrooms were shown to be effective in the treatment of TB in research published in the Journal of ethnopharmacology. According to a study, shiitake mushroom polysaccharide can promote the synthesis of interferon-gamma (IFN-γ), a key immune system protein that aids in the battle against tuberculosis.[43] Lentinan's protective effects are mediated mostly by T helper type 1 (Th1) cells and macrophages, generating a delayed-type sensitivity response in the host of particular importance is Lentinan-induced activation of macrophages for *M. Tuberculosis* killing. Macrophages are the principal cell type engaged in the initial absorption of *M. tuberculosis* and the main effector cells of TB cell mediated immunity.[44] Furthermore, shiitake mushroom extract has been demonstrated to have anti-inflammatory and antioxidant effects, which can help to minimize TB lung damage and to increase overall survival rates in TB patients. More research is needed, however, to completely understand the potential of shiitake mushrooms in treating resistant TB, as well as to identify proper dose and delivery.

5.13. Pleurotus ostreatus *Jacq. Fr (Kumm)*

Oyster mushrooms (*Pleurotus ostreatus*) are a species of edible fungus that is commonly grown and consumed worldwide. It has long been used in Chinese and Japanese medicine to cure a variety of diseases, including tuberculosis (TB). They are also known to have therapeutic effects. They have long been used in Chinese and Japanese medicine to treat a variety of diseases, including high blood pressure and excessive cholesterol. A few *in-vitro* studies have revealed that oyster mushroom extract may have anti-inflammatory, antioxidant, and immune-boosting effects that might aid in the treatment of TB. More study is needed to understand the particular processes through which oyster mushroom extract may aid in the treatment of resistant TB, as well as to identify suitable doses and delivery.[45] The precise method through which oyster mushrooms may aid in the treatment of multidrug-resistant tuberculosis (MDR-TB) remains unknown. *In-vitro* studies, however, have revealed that the active chemicals in oyster mushrooms, such as polysaccharides, may have immunomodulatory effects that might help enhance the immune system and combat MDR-TB infections. Another study discovered that oyster mushroom extract can limit the development of *M. tuberculosis*, the bacteria responsible for tuberculosis, and may have promise as an adjuvant therapy in the treatment of MDR-TB.[46] According to a study oyster mushroom polysaccharides can boost the production of interferon-gamma (IFN-γ), a key immune system protein that aids in the battle against TB. These mushrooms are richest sources of vitamin-D. As per this research, it was discovered a considerable rise in IFN-γ levels but not in IL-4 or IL-10 levels. Vitamin D therapy resulted in a substantial change in IFN-α production in TB patients with VDD at baseline, according to research. *In vitro* studies also shown that 25(OH)D3 treatment of vitamin D deficient serum recovered IFN-γ. The level of cathelicidin LL-37 changed significantly and link between LL-37, IFN-γ, and 25(OH)D levels. This suggested that a higher vitamin D status raises the amount of IFN-γ mediated cathelicidin LL-37.[47] However, more study is needed to completely understand the potential of oyster mushrooms in the treatment of MDR-TB, as well as to identify the proper dose and delivery. It's also worth noting that research on oyster mushrooms in the treatment of MDR-TB is still in its early stages, and further study is needed before drawing any conclusions.

5.14. *Polyporus biformis Fr*

Polyporus biformis, also known as Trichoderma biforme, is a fungus species in the Polyporaceae family. It is also known as

a "double-form polypore." The fungus grows on dead or dying hardwood trees, mainly oak, and beech, throughout Europe and North America. It has a rough, woody peel and is often dark brown or black in color. Although the fungus is edible and not widely consumed as a food source. Some studies indicate that *P. biformis*, a fungus, may have therapeutic potential for tuberculosis (TB). Polysaccharides and triterpenoids derived from this fungus exhibit an antibacterial effect against the bacteria that causes tuberculosis, *M. tuberculosis*, according to research. These substances have been demonstrated in laboratory experiments to suppress bacterial growth.[48] The fungus also has immunomodulatory qualities, which means it can assist the immune system fight off the TB-causing bacteria. However, these discoveries are only in the early phases of study, and much more research is required to evaluate the clinical safety and efficacy of *P. biformis* as a therapy for tuberculosis in people.

5.15. Isochrysis galbana *parke*

Isochrysis is a genus of microalgae that is extensively used as a food source for aquatic creatures such as zooplankton, oysters, and fish larvae in aquaculture and marine research. They are also used to make biofuels and provide omega-3 fatty acids for human consumption. They've also been examined for their possible use in heavy metal pollution bioremediation and as a source of pigments for the food and cosmetic industries. It is also having antimicrobial properties and in addition, research has also found that it contains compounds such as fucoxanthin and phycobilin proteins which are known to have antimicrobial, anti-inflammatory, and antioxidant properties that could be helpful in treating TB.[49] According to research on Isochrysis, polysaccharides generated by this microalga may have a direct inhibitory effect on *M. tuberculosis* development, most likely by interfering with the bacteria's cell wall and membrane. Furthermore, some research suggests that the pigments generated by Isochrysis may have antioxidant qualities that help to protect host cells from the harm caused by TB infections.[50] It should be noted that research on the use of Isochrysis as a treatment for multidrug-resistant tuberculosis (MDR-TB) is still in its early phases, and additional study is required to understand the safety and efficacy of employing this microalga as a treatment for MDR-TB.

5.16. *Arthrospira plantensis Gomont*

A. platensis also known as spirulina', which is a blue-green algae that is commonly used as a nutritional supplement. It is high in protein, vitamins, and minerals, as well as antioxidants. According to several research, spirulina may offer health advantages such as lowering inflammation and relieving symptoms of certain illnesses such as allergies. More study, however, is required to confirm these possible advantages. The antibacterial activity of the spirulina methanolic extract was greater than that of other extracts, with inhibition zones ranging from 17 to 22 mm at a concentration of 10 mg/mL. MIC results verified these findings, with Spirulina methanolic extract having the lowest MIC (1–2 mg/mL) against tested bacteria when compared to other extracts.[51] Spirulina extract has been discovered to prevent the growth of *M. Tuberculosis* and to enhance the action of anti-tubercular medicines.[52] However, the exact mechanism through which *A. platensis* may exercise its possible therapeutic benefits on multidrug-resistant tuberculosis (MDR-TB) remain unclear.

5.17. *Polysiphonia virgata C. Agardh*

Red algae (Rhodophyta) have been studied for their possible therapeutic effects, and their antibacterial activity has been demonstrated. Several types of red algae have been studied to see if they could be used to treat tuberculosis (TB). Certain compounds present in red algae, such as fucoidans and sulfated polysaccharides, have been demonstrated in certain studies to have anti-tubercular action and may be effective in the treatment of MDR-TB. A variety of long-chain fatty acids as the primary antimycobacterial chemicals, including oleic acid, linoleic acid, lauric acid, and myristic acid were also extracted from Polysiphonia virgata. These chemicals have been proven to limit the development of *M. tuberculosis*, the organism that causes tuberculosis, and may improve the efficacy of currently available TB medications.[53,54] These chemicals is that they may interfere with the bacterial cell wall and cell membrane, making it difficult for the bacteria to live and reproduce. Some red algal chemicals have also been demonstrated to block enzymes required for *M. tuberculosis* growth and survival, which may contribute to their anti-tubercular efficacy. Further, these chemicals demonstrated to induce the production of pro-inflammatory cytokines, which may aid in activating immune cells and promoting *M. tuberculosis* clearance from the host. More study is required to understand the precise mechanisms of action of red algae chemicals and to prove their usefulness in the treatment of MDR-TB in humans and their effectiveness in synergistic therapy with existing anti-TB medications.[55,56] At minimal inhibitory concentrations (MIC) of 25 g/mL, lauric acid, myristic acid, and linoleic acid all showed 100% inhibition; at MIC values of 50 g/mL, all three acids showed 100% inhibition. At 50 g/mL, myristic acid and lauric acid inhibited the enzyme by 90% and 76%, respectively. Linoleic acid inhibited the development of a clinical strain of multidrug-resistant *M. tuberculosis* somewhat 50 μg/mL.[53]

In spite of the limited number of plants discussed in treating the multi drug resistance tuberculosis, the findings of the current comprehensive review hold promise as a foundation upon which to build a new class of drugs for the treatment of tuberculosis. The antimycobacterial activity, safety, and efficacy of the aforementioned plant species warrant further isolation and purification of the bioactive compounds responsible for these properties. The various important bio-sources useful in the antitubercular drug discovery have been mentioned in Table 1.

Table 1 – Biosources useful in antitubercular drug discovery.

S. No	Name of the plant	Family	Traditional values	Antitubercular mechanisms	References
1.	*Curcuma longa* Linn.	Zingiberaceae	Fever, jaundice, chronic diarrhoea, cancer, rheumatoid arthritis, asthma, eye disorders	By inhibiting p65 NF-kappa B, binding to its consensus oligonucleotide and activating NF-kappaB, and inducing apoptosis, curcumin causes a biological response in macrophages that drastically reduces bacterial survival.	57
2.	*Bauhinia vahlii* Wight.	Zingiberaceae	Improves digestion, relieves inflammation, respiratory issues	The antitubercular mechanisms of *Bauhinia vahlii* Wight. is still unclear but the methanolic extracts showed significant activity of 0.05 ± 0.01 to 0.26 ± 0.01 nM against *Mycobacterium tuberculosis*	58
3.	*Acalypha indica* Linn.	Asclepiadaceae	Asthma, bronchitis, blood purification, treating skin conditions like eczema and psoriasis, UTI and anemia	The antitubercular molecular mechanisms of *Acalypha indica* is still unclear and however the aqueous extract showed significant inhibitory activity of 95% on MDR TB strains.	59
4.	*Allium cepa* Linn.	Amaryllidaceae	Asthma, bronchitis, diarrhoea, skin infections, improves vision	The quercetin was isolated from the *A. cepa* and based on the results of molecular docking, quercetin forms the most stable complex with β-lactamase, with a binding energy of −4.80 Kcal/mol, compared to −2.62 Kcal/mol with gyarse A, −3.62 Kcal/mol with 2-trans-enoyl-acyl carrier protein reductase-inhA, and −4.6 Kcal/mol with topoisomerase IV.	59
5.	*Adathodai vasica* Nees.	Fabaceae	Fever, headache, respiratory, cardiovascular disorders, treat alcoholism	*A. vasica* leaf hexane extract yielded two compounds, vasicine acetate and 2-acetyl benzylamine, which strongly inhibited *M. tuberculosis* at 200 and 50 microg/mL, respectively. The molecular mechanisms of the drug is still unclear.	23
6.	*Glycyrrhiza glabra* var. glandulifera.	Marasmiaceae	Lowering cholesterol, Supporting the immune system, cardiovascular health, liver health, digestion, health, skin health.	Isoliquiritigenin, a flavonoid in liquorice, reduces inflammation caused by Mycobacterium TB by inhibiting the Notch1/NF-kB and MAPK signalling pathways.	
7.	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Pleurotaceae	Improves digestion, boosts immune system, lowers cholesterol, Alzheimer's disease	No direct evidences on the inhibition of *M. tuberculosis*	60
8.	*Polyporus biformis* Fr.	Polyporaceae	Treating skin conditions like eczema and psoriasis, UTI, hypertension, diabetes, fever	Biformin, a biguanide discovered in a *P. biformis* culture, has been shown to be antitubercular against *M. tuberculosis* H 37 Rv at a minimum inhibitory concentration (MIC) of 0.56 g/mL.	61
9.	*Isochrysis galbana* parke.	Isochrysidaceae	Respiratory conditions, skin diseases, food supplement for vitamins, omega 3 fatty acids for brain function	The n-hexane extracts of *I. galban* by bioassay-guided fractionation in order to isolate and characterize their antimycobacterial compounds. Polysaccharides has been isolated from these genus	62
10.	*Arthospira plantensis* Linn.	Phormidiaceae	Dietary supplement, lowering inflammation and relieving symptoms of allergies	Unclear mechanisms	–
11.	*Polysiphonia virgata* C. Agardh	Rhodomelaceae	Promotes healthy circulation, lower bad cholesterol and regulates blood sugar levels	Inhibited 0.56 g/mL against *M. smegmatis*.	63

6. Conclusion

The review suggests that phytochemicals derived from plants and fungi may be effective in the treatment of MDR-TB as well as act as adjunct agents and can show synergistic action when used with synthetic drugs. Further, they can reduce the harmful effects of synthetic drugs. The efficacy of phytochemicals in combination with conventional therapeutics has been demonstrated in clinical trials, but their full potential is yet to be explored due to limitations such as unclear mechanisms of action and unknown interactions with the human diet and standard pharmaceuticals. Overcoming these obstacles would require multidisciplinary research involving drug discovery, molecular biology, and clinical trials. However, plant-based medication helps to restore the balance between the host's pro-inflammatory and anti-inflammatory cytokine response, which is often disrupted by bacterial infections. Several plant-derived molecules, such as Allicin, adhatodine, and piperine, share a pharmacophore with new anti-TB drugs currently in clinical trials. The development of phytochemical co-therapy approaches to complement existing TB drugs takes less time and costs less money than the search for a leading anti-TB drug candidate. Scientists are eager to explore these new drugs, and funding agencies are preparing to provide money for research and development as soon as new agents become available. Most of the drugs from natural sources mentioned here are likely to have fewer or no adverse effects, and they are expected to show synergism in combination with existing antitubercular agents. However, before clinical use, the evidence of the safety of such combination needs to be addressed with suitable preclinical studies.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Barberis I, Bragazzi NL, Galluzzo L, Martini M. *The History of Tuberculosis: From the First Historical Records to the Isolation of Koch's Bacillus*. vol. 58. 2017.
2. Ias D. World tuberculosis report 2022: WHO Why in News? https://www.drishtiias.com/printpdf/world-tuberculosis-report-2022-who.
3. Lange C, Dheda K, Chesov D, Mandalakas AM, Udwadia Z, Horsburgh R. *Management of Drug-Resistant Tuberculosis*; 2019. vol. 394 www.thelancet.com/.
4. Saeed DK, Shakoor S, Razzak SA, et al. Variants associated with Bedaquiline (BDQ) resistance identified in Rv0678 and efflux pump genes in Mycobacterium tuberculosis isolates from BDQ naïve TB patients in Pakistan. *BMC Microbiol*. 2022;22(1). https://doi.org/10.1186/s12866-022-02475-4.
5. Hashemian SMR, Farhadi T, Ganjparvar M. Linezolid: a review of its properties, function, and use in critical care. *Drug Des Dev Ther*. 2018;12:1759–1767. https://doi.org/10.2147/DDDT.S164515.
6. Chen Y, Chen J, Zhang S, et al. Novel mutations associated with clofazimine resistance in mycobacterium abscessus. *Antimicrob Agents Chemother*. 2018;62(7). https://doi.org/10.1128/AAC.00544-18.
7. Rifat D, Li SY, Ioerger T, et al. Mutations in fbiD (Rv2983) as a novel determinant of resistance to pretomanid and delamanid in mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2021;65(1). https://doi.org/10.1128/AAC.01948-20.
8. Kadura S, King N, Nakhoul M, et al. Systematic review of mutations associated with resistance to the new and repurposed Mycobacterium tuberculosis drugs bedaquiline, clofazimine, linezolid, delamanid and pretomanid. *J Antimicrob Chemother*. 2020;75(8):2031–2043. https://doi.org/10.1093/jac/dkaa136.
9. Wu SH, Chan HH, Hsiao HC, Jou R. Primary bedaquiline resistance among cases of drug-resistant tuberculosis in Taiwan. *Front Microbiol*. 2021;12. https://doi.org/10.3389/fmicb.2021.754249.
10. Pi R, Liu Q, Jiang Q, Gao Q. Characterization of linezolid-resistance-associated mutations in Mycobacterium tuberculosis through WGS. *J Antimicrob Chemother*. 2019;74(7):1795–1798. https://doi.org/10.1093/jac/dkz150.
11. Reichmuth ML, Hömke R, Zürcher K, et al. Natural polymorphisms in Mycobacterium tuberculosis conferring resistance to delamanid in drug-naive patients. *Antimicrob Agents Chemother*. 2020;64(11). https://doi.org/10.1128/AAC.00513-20.
12. Richard M, Gutiérrez AV, Viljoen A, et al. Mutations in the MAB_2299c TetR regulator confer cross-resistance to Clofazimine and Bedaquiline in Mycobacterium abscessus. *Antimicrob Agents Chemother*. 2019;63(1). https://doi.org/10.1128/AAC.01316-18.
13. Gautam R, Saklani A, Jachak SM. Indian medicinal plants as a source of antimycobacterial agents. *J Ethnopharmacol*. 2007;110(2):200–234. https://doi.org/10.1016/j.jep.2006.12.031.
14. Barua N, Buragohain AK. Therapeutic potential of curcumin as an antimycobacterial agent. *Biomolecules*. 2021;11(9). https://doi.org/10.3390/biom11091278.
15. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The essential medicinal chemistry of curcumin. *J Med Chem*. 2017;60(5):1620–1637. https://doi.org/10.1021/acs.jmedchem.6b00975.
16. Bai X, Oberley-Deegan RE, Bai A, et al. Curcumin enhances human macrophage control of Mycobacterium tuberculosis infection. *Respirology*. 2016;21(5):951–957. https://doi.org/10.1111/resp.12762.
17. Marini E, Di Giulio M, Magi G, et al. Curcumin, an antibiotic resistance breaker against a multiresistant clinical isolate of Mycobacterium abscessus. *Phytother Res*. 2018;32(3):488–495. https://doi.org/10.1002/ptr.5994.
18. Boonphong S, Puangsombat P, Baramee A, Mahidol C, Ruchirawat S, Kittakoop P. Bioactive compounds from Bauhinia purpurea possessing antimalarial, antimycobacterial, antifungal, anti-inflammatory, and cytotoxic activities. *J Nat Prod*. 2007;70(5):795–801. https://doi.org/10.1021/np070010e.
19. Nguyen TT, Ketha A, Hieu HV, Tatipamula VB. In vitro antimycobacterial studies of flavonols from Bauhinia vahlii Wight and Arn. *3 Biotech*. 2021;11(3). https://doi.org/10.1007/s13205-021-02672-4.
20. *The Ayurvedic Pharmacopoeia of India Part-I Volume-IX Government of India Ministry of Ayush 2016*. 1st ed. Ghaziabad: Pharmacopoeia Commission for Indian Medicine & Homoeopathy; 2016. सत्यमे व जयते.
21. Shenoy RP. *Effects of Selective Medicinal Plants against Multi Drug Resistance Mycobacterium Tuberculosis Strains*. vol. 13. 2013. https://www.researchgate.net/publication/286112354.
22. Yang XW, Li SM, Shen YH, Zhang WD. REVIEW Phytochemical and Biological Studies of Abies Species.

23. Shamsuddin T, Alam MS, Junaid Md, et al. Adhatoda vasica (Nees.): a review on its botany, traditional uses, phytochemistry, pharmacological activities and toxicity. *Mini-Rev Med Chem*. 2021;21(14). https://doi.org/10.2174/1389557521666210226152238.
24. Shoaib A. A systematic ethnobotanical review of Adhatoda vasica (L.), Nees. *Cell Mol Biol*. 2021;67(4):248–263. https://doi.org/10.14715/CMB/2021.67.4.28.
25. Ignacimuthu S, Shanmugam N. Antimycobacterial activity of two natural alkaloids, vasicine acetate and 2-acetyl benzylamine, isolated from Indian shrub Adhatoda vasica Ness. leaves. *J Biosci*. 2010;35(4):565–570. https://doi.org/10.1007/s12038-010-0065-8.
26. Mishra S, Khatri M. Inhibition of extracellular and intracellular survival of mycobacterial strains by alkaloids extracted from Justicia adhatoda leaves. *Eur J Integrat Med*. 2022. https://doi.org/10.21203/rs.3.rs-1008118/v3. Published online.
27. Gupta VK, Fatima A, Faridi U, et al. Antimicrobial potential of Glycyrrhiza glabra roots. *J Ethnopharmacol*. 2008;116(2):377–380. https://doi.org/10.1016/j.jep.2007.11.037.
28. Vaya J, Belinky PA, Aviram M. Antioxidant constituents from licorice roots: isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radial Biol Med*. 1997;23.
29. Mitscher LA, Has PARK T sg, Clark D, Beal JL. A K T I Hvf I CRO B I A I, IS 0 F LXVA4 N 0 I D S X N D RE L-ITE D Substances from GLYCYRRHIZA GLABRA L. YAIR. TYPICA.
30. Ghosh S, Saha S. Tinospora cordifolia: one plant, many roles. *Ancient Sci Life*. 2012;31(4). https://doi.org/10.4103/0257-7941.107344.
31. Gupta PK, Chakraborty P, Kumar S, et al. G1-4A, a polysaccharide from Tinospora cordifolia inhibits the survival of Mycobacterium tuberculosis by modulating host immune responses in TLR4 dependent manner. *PLoS One*. 2016;11(5). https://doi.org/10.1371/journal.pone.0154725.
32. Selva Elsee GT, Sathiya HX. Anti tuberculosis potential of Siddha Herbs-a review. www.ejpmr.com.
33. Herbst S, Schaible UE, Schneider BE. Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. *PLoS One*. 2011;6(5). https://doi.org/10.1371/journal.pone.0019105.
34. Alajmi MF, Mothana RA, Al-Rehaily AJ, Khaled JM. Antimycobacterial activity and safety profile assessment of alpinia galanga and Tinospora cordifolia. *Evid base Compl Alternat Med*. 2018;2018. https://doi.org/10.1155/2018/2934583.
35. Suja S, Chinnaswamy P. Inhibition of in Vitro Cytotoxic Effect Evoked by Alpinia Galanga and Alpinia Officinarum on PC-3 Cell Line.
36. Published online. *Smilax Zeylanica Linn: Pharmacognostic, Taxonomical and Phytochemical Status*. 2022. https://doi.org/10.37591/RRJoHS.
37. Nandy S, Singh J, Pandey DK, Dey A. Hemidesmus indicus L. Br.: critical assessment of in vitro biotechnological advancements and perspectives. *Appl Microbiol Biotechnol*. 2020;104(20):8517–8548. https://doi.org/10.1007/s00253-020-10851-1.
38. Kundu A, Mitra A. Evaluating tyrosinase (monophenolase) inhibitory activity from fragrant roots of Hemidesmus indicus for potent use in herbal products. *Ind Crops Prod*. 2014;52:394–399. https://doi.org/10.1016/j.indcrop.2013.10.053.
39. Kianian F, Marefati N, Boskabady M, Ghasemi SZ, Boskabady MH. Pharmacological properties of allium cepa, preclinical and clinical evidences; a review. *Iran J Pharm Res (IJPR)*. 2021;20(2):107–134. https://doi.org/10.22037/ijpr.2020.112781.13946.
40. Adaikkappan P, Kannapiran M, Anthonisamy A. Anti-mycobacterial activity of Withania somnifera and Pueraria tuberosa against Mycobacterium tuberculosis H 37 Rv. *J Acad Indus Res*. 2012;1(4):153.
41. Chakraborty AJ, Uddin TM, Matin Zidan BMR, et al. Allium cepa: a treasure of bioactive phytochemicals with prospective health benefits. *Evid base Compl Alternative Med*. 2022:2022. https://doi.org/10.1155/2022/4586318.
42. Bharti R, Chopra BS, Raut S, Khatri N. Pueraria tuberosa: a review on traditional uses, pharmacology, and phytochemistry. *Front Pharmacol*. 2021;11. https://doi.org/10.3389/fphar.2020.582506.
43. Crespo H, Guillén H, de Pablo-Maiso L, et al. Lentinula edodes β-glucan enriched diet induces pro-and anti-inflammatory macrophages in rabbit. *Food Nutr Res*. 2017;61. https://doi.org/10.1080/16546628.2017.1412791.
44. Roszczyk A, Turło J, Zagożdżon R, Kaleta B. Immunomodulatory properties of polysaccharides from Lentinula edodes. *Int J Mol Sci*. 2022;23(16). https://doi.org/10.3390/ijms23168980.
45. Lesa KN, Khandaker MU, Rashed Iqbal F Mohammad, et al. Nutritional value, medicinal importance, and health-promoting effects of dietary mushroom (Pleurotus ostreatus). *J Food Qual*. 2022:2022. https://doi.org/10.1155/2022/2454180.
46. Shree AK, Balamurugan TSB, Babu GRN. Phytochemical, antioxidant and antitumor activity of edible mushroom pleurotus ostreatus. *Int J Adv Res Biol Sci*. 2016;3(9):170–177. https://doi.org/10.22192/ijarbs.
47. Fabri M, Stenger S, Shin DM, et al. Vitamin D is required for IFN-γ-mediated antimicrobial activity of human macrophages. *Sci Transl Med*. 2011;3(104). https://doi.org/10.1126/scitranslmed.3003045.
48. Zjawiony JK. Antitubercular Activity of Mushrooms (Basidiomycetes) and Their Metabolites.
49. Ruiz-Domínguez MC, Salinas F, Medina E, et al. Supercritical fluid extraction of fucoxanthin from the diatom phaeodactylum tricornutum and biogas production through anaerobic digestion. *Mar Drugs*. 2022;20(2). https://doi.org/10.3390/md20020127.
50. Prakash S, Bhimba V. Natural Product Radiance Pharmaceutical Development of Novel Microalgal Compounds for Mdr Mycobacterium Tuberculosis.
51. Martin SJ, Baskaran UL, Vedi M, Sabina EP. Attenuation of anti-tuberculosis therapy induced hepatotoxicity by Spirulina fusiformis, a candidate food supplement. *Toxicol Mech Methods*. 2014;24(8):584–592. https://doi.org/10.3109/15376516.2014.956910.
52. Abdel-Moneim AME, El-Saadony MT, Shehata AM, et al. Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. *Saudi J Biol Sci*. 2022;29(2):1197–1209. https://doi.org/10.1016/j.sjbs.2021.09.046.
53. Mishra S, Khatri M, Mehra V. Assessing the antimycobacterial activity of the bioactive fractions of the indian medicinal plant-justicia adhatoda l. *Chemical Biology Letters*. 2021;8(2).
54. Barua N, Buragohain AK. Therapeutic potential of curcumin as an antimycobacterial agent. *Biomolecules*. 2021;11(9). https://doi.org/10.3390/biom11091278.
55. Saravanakumar DEM, Folb PI, Campbell BW, Smith P. Antimycobacterial activity of the red alga *Polysiphonia virgata*. *Pharm Biol*. 2008;46(4):254–260. https://doi.org/10.1080/13880200701739413.
56. Kaur R, Kaur H. Antitubercular activity and phytochemical screening of selected medicinal plants. *Orient J Chem*. 2015;31(1):597–600. https://doi.org/10.13005/ojc/310176.
57. Nguyen TT, Ketha A, Hieu HV, Tatipamula VB. In vitro antimycobacterial studies of flavonols from Bauhinia vahlii

Wight and Arn. *3 Biotech*. 2021;11(3). https://doi.org/10.1007/s13205-021-02672-4.
58. Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM, et al. Anti-Tuberculosis Activity of Selected Medicinal Plants against Multi-Drug Resistant Mycobacterium Tuberculosis Isolates. http://journals.lww.com/ijmr.
59. Nair SS, Gaikwad SS, Kulkarni SP, Mukne AP. Allium sativum constituents exhibit anti-tubercular activity in vitro and in RAW 264.7 mouse macrophage cells infected with Mycobacterium tuberculosis H37Rv. *Phcog Mag*. 2017;13(50):S209–S215. https://doi.org/10.4103/pm.pm_435_16.
60. Castillo TA, Lemos RA, Pereira JRG, Alves JMA, Teixeira MFS. Mycelial growth and antimicrobial activity of Pleurotus species (Agaricomycetes). *Int J Med Mushrooms*. 2018;20(2). https://doi.org/10.1615/IntJMedMushrooms.2018025477.
61. Zjawiony JK. Antitubercular activity of mushrooms (Basidiomycetes) and their metabolites. *Nat Prod Commun*. 2007;2(3). https://doi.org/10.1177/1934578x0700200314.
62. Saravanakumar DEM, Folb PI, Campbell BW, Smith P. Antimycobacterial activity of the red alga Polysiphonia virgata. *Pharm Biol*. 2008;46(4). https://doi.org/10.1080/13880200701739413.
63. Saravanakumar DEM, Folb PI, Campbell BW, Smith P. Antimycobacterial activity of the red alga Polysiphonia virgata. *Pharm Biol*. 2008;46(4):254–260. https://doi.org/10.1080/13880200701739413.

Indian Journal of Tuberculosis 71 (2024) S130–S135

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Review article

Favorable clinical outcomes and anti-mycobacterial efficacy of pretomanid in patients with highly resistant tuberculosis: A review

Tanishq Joshi [a], Parminder Nain [b], Prajwal Bhamra [a], Jaspreet Kaur [b,*]

[a] Department of Pharmacy Practice, MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala 133207, India
[b] Department of Pharmacy Practice, CT Institute of Pharmaceutical Sciences, Shahpur, Jalandhar, Punjab 144020, India

ARTICLE INFO

Keywords:
Tuberculosis
MDR-TB
XDR-TB
Pretomanid

ABSTRACT

Rising cases of drug resistance of mycobacterium species are one of the biggest concerns when the goal is to eradicate TB (Tuberculosis) from the world by the year 2030. A limited number of treatment options as MTB (*Mycobacterium tuberculosis*) is getting resistant to anti-mycobacterial drugs either due to a patient's non-compliance towards treatment regimen or if a patient is infected by drug-resistant species of MTB. This review aims to assess the effectiveness of pretomanid, a recently approved drug for the treatment of extensively drug-resistant TB. A thorough search of databases like PubMed, Cochrane library, CDC, Research Gate, and Google scholar was used in order to find case reports and clinical trials providing data on the efficacy of pretomanid in different drug regimens. According to research trials conducted, the drug appears to be efficacious, safe, and well-tolerable. Only headache was the most frequently observed adverse drug event, and a high dose-related increase in serum creatinine level was seen, which came to normal after the drug was discontinued.

1. Introduction

Tuberculosis (TB) is a bacterial infection that typically entails the lungs and is caused by a pathogen named *Mycobacterium tuberculosis* (MTB). It holds second place in the list of top deadliest pathogens caused by COVID-19, bred by the SARS-CoV-2 virus, and worldwide it is at 13th place as a leading cause of death. According to the World Health Organization, 10 million people throughout the globe will be infected with tuberculosis (TB) in the year 2020.[1] Dissemination of TB among different populations consists of 1.1 million children, 3.3 million women, and 5.6 million men. In the year 2020, a total of 1.5 million people lost their lives to TB, of which 214,000 were HIV/AIDS-positive patients. TB is present in all countries but is curable if the infected patient has good compliance with their treatment regimen. In the year 2020, a contribution of 86% of new cases was from 30 significant TB-burdened countries, of which key contributors were China, Pakistan, Indonesia, Bangladesh, Nigeria, South Africa, and the Philippines, with a percentage of 67%.[2] Multidrug-resistant TB (MDR-TB) is a public health calamity. Approximately 490,000 people worldwide were detected with MDR-TB and 110,000 people with specific drug resistance (Rifampin) TB in the year 2016. About 6.2% of these cases were estimated to be extensively drug-resistant TB.[3]

Coughing for more than 3 weeks, coughing sputum (phlegm) or blood, and chest pain are the most common clinical manifestations of tuberculosis. Other clinical manifestations include weight loss, decreased or no appetite, night sweating, fatigue, chills, and fever.[2] Medical conditions that weaken the immune system in patients, such as HIV/AIDS, diabetes, severe kidney disease, substance abuse, silicosis, organ transplants, and head and neck cancers, are all risk factors. Patients on treatment regimens for rheumatoid arthritis and Crohn's disease are also at risk of developing TB. TB gets transmitted from a person with active TB to a normal person through cough droplets, sneezing, singing, or talking.[4]

2. Materials & methods

Different databases *i.e.* Scopus, PubMed, Science Direct, and Google Scholar were used to identify relevant articles only in the English language published through January 30, 2020, for literature review Searched terms included pathophysiology of TB, reactivation of infection, treatment guidelines of drug-sensitive TB, and drug resistance case studies of anti-tubercular treatment resistance, Pretomanid. The search resulted in 267 total articles. We also included case reports, case series, and review papers due to the lack of RCTs. For inclusion, the authors

* *Corresponding author*. Tel.: 8059930164.
E-mail address: preetisidana@gmail.com (J. Kaur).

https://doi.org/10.1016/j.ijtb.2023.09.011
Received 27 June 2022; Received in revised form 16 September 2023; Accepted 22 September 2023
Available online 27 October 2023

separately assessed the titles and abstracts. Additional relevant articles related to pretomanid were evaluated from the review of citations referenced journals.

2.1. Pathophysiology of TB

Inhalation of aerosolized droplets of *Mycobacterium tuberculosis* (MTB) from an infected person leads to certain outcomes in the human body, like immediate clearance of the organism, latent infection, primary infection, or activation of the latent phase. MTB spreads the infection to healthy lungs through aerosolized droplets ranging from 5 to 10 microns that reach alveolar sacs.[5] Macrophages are part of our body's defense system and are normally present in alveolar sacs to perform phagocytosis on any foreign microbe in order to protect our lungs. If macrophages fail to eliminate the bacteria, then it replicates inside cells, and eventually kills the cells. Macrophages produce chemokines and cytokines in response to MTB, leading to the accumulation of phagocytes like monocytes, neutrophils, and other macrophages. This eventually results in the formation of a nodular granulomatous called the tubercle.[6] If bacteria replication is not halted, tubercles increase in size and MTB invades local lymph nodes, causing lymphadenopathy, one of the clinical manifestations of primary tuberculosis. Enlarged tubercle in lung parenchyma and its lymph node invasion together form ghon complex. The human body develops cell-mediated immunity in a period of 2–6 weeks after infection. Failure of effective cell-mediated response and repair of tissue leads to progressive lung damage. Reactive oxygen species, nitrogen intermediate, TNF alpha, and other cytotoxic cells contribute to the formation of the TB lesion known as caseating necrosis.[7] Unchecked bacterial growth of MTB leads to its systemic spread with lesions resembling millets, which is known as miliary TB. MTB can also be spread by erosion of a caseous lesion into the lungs' airways, which leads to the spread of infection by the host. Chronic infection is associated with repeated episodes of healing by the formation of fibrotic lesions and tissue breakdown.[8]

2.2. Reactivation of infection

The proliferation of dormant bacteria from a previous infection leads to the reactivation of the TB infection. In individuals with latent infection and no underlying medical condition, the chance of reactivation is 5–10%. Reactivation of infection is associated with immunosuppression, either due to any medical condition or due to immunosuppressive therapy.[9] Although host factors responsible for the maintenance of infection in the latent phase are uncertain, reactivation of TB is mostly localized with little involvement of regional lymph nodes and less caseous necrosis. The lesion occurs at lung apices, and invasion of infection to other organs occurs only if the host is severely immune-compromised. Protection against subsequent TB exposure has been observed in patients with latent TB.[8]

2.3. Treatment guidelines of drug-susceptible TB (centre for disease control and prevention)

The prime focus of tuberculosis treatment is on curing the infected individual and minimizing the spread of infection (MTB) for successful tuberculosis treatment that will benefit both the patients as well as the community (Table 1). The main objectives of the treatment are to reduce the number of rapidly growing bacilli, eliminate persisting MTB, and prevent the occurrence of drug resistance.[10,11]

2.4. Drug resistance

Patients previously treated with this drug regimen and were unable to complete the course of treatment due to any reason or were in contact with a person who has drug-resistant MTB, there is the possibility of MTB acquiring resistance against these drugs.[12] If MTB acquires resistance to one of the first-line drugs, it is referred to as drug-resistant TB. When MTB acquires resistance against two drugs from the first-line regimen, (at least against isoniazid or rifampin), it is referred to as MDR-TB (multi-drug-resistant – TB). Where MTB acquires resistance to isoniazid, and rifampin, with one drug from the 2nd line injectable (capreomycin, amikacin, or kanamycin) and one fluoroquinolone, it is referred to as XDR-TB (extensively drug resistant-TB). According to a recent study, there were an estimated 450,000 cases of multidrug-resistant tuberculosis (MDR-TB) in 2021, an increase of 3.1% from 2020. India, Russia, and Pakistan accounted for 42% of the global cases, with India accounting for 26%, Russia for 8.5%, and Pakistan for 7.9% of cases.[13] Patients with multi-drug-resistant TB have high levels of pre-existing drug resistance and extensive drug resistance, with the former at 9% and the latter at 26%, of which the prevalence of fluoroquinolones (FQN) and Second-line Injectable Drugs (LSID) was 27% and 11% respectively. Furthermore, MDR-TB patients are resistant to novel TB drugs in 4%–5% of cases.[14] Treatment of drug-resistant TB can be uncertain, and an inappropriate regimen or management can be fatal.

Table 1
Treatment guidelines of drug-susceptible TB.

Regimen	Intensive phase		Continuation phase		Range of total doses	Comments (a,b)
	Drug	Interval and dose (Minimum duration)	Drugs	Interval and dose (a) (Minimum duration)		
1	INH RIF PZA EMB	7 d/wk for 56 doses (8 wk), or 5 d/wk for 40 doses (8 wk)	INH RIF	7 d/wk for 126 doses (18 wk), or 5 d/wk for 90 doses (18 wk)	182–130	This is the preferred regimen for patients with newly diagnosed pulmonary tuberculosis.
2	INH RIF PZA EMB	7 d/wk for 56 doses (8 wk), or 5 d/wk for 40 doses (8 wk)	INH RIF	3 times weekly for 54 doses (18 wk)	110–94	Preferred alternative regimen in situations in which more frequent DOT during continuation phase is difficult to achieve.
3	INH RIF PZA EMB	3 times weekly for 24 doses (8 wk)	INH RIF	3 times weekly for 54 doses (18 wk)	78	Use regimen with caution in patients with HIV and/or cavitary disease. Missed doses can lead to treatment failure, relapse, and acquired drug resistance.
4	INH RIF PZA EMB	7 d/wk for 14 doses then twice weekly for 12 doses	INH RIF	Twice weekly for 36 doses (18 wk)	62	Do not use twice-weekly regimens in HIV-infected patients or patients with smear-positive and/or cavitary disease. If doses are missed, then therapy is equivalent to once weekly, which is inferior.

(a) After the completion of 2-month therapy, if patient still had positive culture and cavitation on initial chest x-ray, then on the basis of expert opinion patient should receive a continuation phase of 7-month.[10]
(b) Patients receiving INH are at the risk of neuropathy should receive 25–50 mg/day dose of pyridoxine.[10]

So, one should refer to a physician expert in treating TB infection and should always maintain good compliance with their treatment regimen.[12]

Multidrug regimens should always be employed for the treatment of MDR-TB/XDR-TB, by including the drugs to which the patient's isolate is susceptible to and omitting the once to which the isolate is resistant. Earlier, fluoroquinolones tend to be the most potent anti-TB agents in a multidrug-resistant tuberculosis (MDR-TB) regimen, in which the later-generation fluoroquinolones (moxifloxacin and levofloxacin) have shown more pronounced effect and were significantly associated with cure rather than an earlier-generation fluoroquinolones. Patients with MDR-TB were suggested to undergo an intensive phase treatment of eight months consisting of at least four second-line anti-TB drugs that are likely to be effective including an injectable anti-TB drug, as well as pyrazinamide.[16] But due to the escalation of fluoroquinolones resistance cases in MDR-TB patients, in December 2022, World Health Organization has published a recent update regarding the treatment of drug-resistant tuberculosis (DR-TB) by releasing a new treatment guideline for the treatment of multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) which recommends the use of 6-month combination regimen with bedaquiline, pretomanid, linezolid (600 mg), and moxifloxacin (BPaLM) therapy rather than 9-month or longer regimens (18 months) and 9-month regimen is suggested instead of longer regimens in fluoroquinolones -susceptible MDR/RR-TB.[17]

2.4.1. Case studies of anti-TB therapy resistance

Case 1. A 27-year-old female patient was first diagnosed and treated for MTB with first-line drugs (HRZE) for six months (2HRZE; 4HR) in the year 2009 and was cured. In the year 2013, a relapse of MTB occurred and she failed her second treatment of drugs (HERZS) of 8 months (2HRZES; 1HRZE; 5HR). Later, she was diagnosed with the Human Immunodeficiency Virus (HIV). They began her treatment for HIV with (tenofovir; emtricitabine; efavirenz). In the year 2014, she was admitted to a specialized unit at UTH for chronic TB. The second line of TB drugs (ofloxacin, ethionamide, kanamycin) was started; a test for anti-mycobacterial agent resistance was performed simultaneously and found that MTB is rifampin resistant. The same treatment of second-line anti-TB drugs continued without any significant improvement. In June 2015, they tested her sputum culture and it was found to be resistant to fluoroquinolones. Drugs for pre-XDR-TB were not available at that time. She was admitted again in December 2015, where she received an irrational treatment regimen (amoxicillin with clavulanic acid; erythromycin; kanamycin; and cycloserine) with irregular monitoring of therapeutic response. She was tested again for sputum culture sensitivity in August 2016 and came out positive for having resistance against anti-tubercular agents.[18]

Case 2. A 47-year-old female patient was diagnosed with tuberculosis in the year 2014 and was HIV-negative. She started her treatment for MTB with first-line drugs (2HRZE; 4HR), but she wasn't able to complete treatment because of the unavailability of medication at her healthcare center. In 2015, she got sick again with constant weight loss and cough. Her sputum got tested positive for acid-fast bacilli test with chest radiographs indicating bilateral dissemination associated with a cavity on the right upper lobe of the lungs. In January 2016, she started with her MDR treatment regimen (2KOEtZ; 4OEtZ) and was discharged in June 2016, after her sputum smear tested negative for MTB. She was not in contact with any MDR-TB infected patient except patient no.1 with whom she shared the same ward between Jan–Jun 2016. Her spoligotype changed from MTB family 4 to family 1 (identical to patient 1) in between those periods. All the above suggests super-infection by nosocomial spread from patient one to patient two. In August 2016, both of the patients were diagnosed with XDR-TB. Then in May 2017, both patients got their treatment: bedaquiline, delamanid, para-aminosalicylic acid, linezolid, clofazimine (2Bdq, Dlm, PAS, Lzd, Cfz, Z, H; 4Bdq, Lzd, Cfz, Z).[18]

2.5. Pretomanid

A new anti-mycobacterial agent was approved recently by the US FDA for the treatment of XDR-TB. It is a nitroimidazooxazine derivative and is given either in the BPaMZ (bedaquiline, pretomanid, moxifloxacin) regimen or in the BPaL (bedaquiline, pretomanid, linezolid) regimen. Both of these regimens were developed by a global alliance of TB drug development (TB alliance) licenced by Novartis. TB alliance has given manufacturing license to Mylan and commercially will be available as BPaL and BPaMZ regimen. License is exclusive to Mylan in high income countries and non-exclusive in low-middle income countries.[19]

2.5.1. Mechanism of action

Non-Replicating Bacteria- Pretomanid undergoes nitro-reduction within MTB by deazaflavin-dependent nitro-reductase (Ddn). Its activation within anaerobic non-replicating bacilli produces a highly reactive intermediary nitro radical anion that interacts with cellular constituents and interrupts cellular respiration. Non-Replicating Bacteria-Pretomanid undergoes nitro-reduction within MTB by deazaflavin-dependent nitro-reductase (Ddn). Its activation within anaerobic non-replicating bacilli produces highly reactive intermediate nitro radical anions which interact with cellular constituents and interrupt cellular respiration.[20]

2.5.2. Replicating bacteria

Pretomanid has the potency to inhibit the biosynthesis of lipids and proteins without affecting the synthesis of nucleic acids. It inhibits the basic component synthesis of the lipid bilayer cell wall known as keto mycolic acid by preventing the oxidative transformation of its precursors.[21] It triggers pentose sugar pathways that lead to the gathering of phosphate sugars, which further leads to the dangerous accumulation of methylglyoxal. Accumulation of this highly reactive ketoaldehyde leads to the glycation of nucleic acids and proteins following cellular arrest.[22]

2.5.3. Pharmacokinetics

Absorption-In a healthy individual, after an oral dose of 50–750 mg, bioavailability was observed to be approximately 55–80% with a T_{max} of 4–5 hours. A plateau in systemic concentration occurs after a single 1000 mg dose or more. Multiple-dose administration showed an accumulation factor of 2.[23]

2.5.4. Distribution

Pretomanid is well distributed with a V_d of 180L in a fasting state and 92L in a fed state and also invades the CNS (central nervous system). The protein binding of this drug with human albumin was found to be 86.4%.[24]

2.5.5. Metabolism

Pretomanid is metabolized by different oxidative and reductive pathways, out of which 20% of pretomanid is metabolized by the liver enzyme CYP3A4.[25]

2.5.6. Excretion

The half-life of pretomanid is 16 hours during a steady-state, 16.9 hours during fasting, and 17.4 hours during a fed state. This drug has shown clearance of 7.6L/hr. during fasting and 3.9L/hr. during a fed state. Most of the drug gets excreted as metabolites due to its extensive metabolism. The amount of dose excreted in urine is 53% and 38% in feces.[23]

2.5.7. Contraindications

A portion of pretomanid is metabolized by the hepatic microsomal enzyme CYP3A4, all strong to moderate inducers such as rifampin, phenytoin, and glucocorticoids must be avoided. Pretomanid is used in combination with linezolid and bedaquiline. In patients having

contraindications against linezolid and bedaquiline, pretomanid is also contraindicated which includes Drug-Sensitive (DS) tuberculosis, Latent infection due to *Mycobacterium tuberculosis*, Extra-pulmonary infection due to *Mycobacterium tuberculosis*, MDR-TB that is not treatment-intolerant or non-responsive to standard therapy. TB resistant to isoniazid and rifampin, who are responsive to standard therapy and not treatment-intolerant with known resistance to any combinations.[25,26]

2.5.8. Dosing

Pretomanid 200 mg, one tablet orally, once daily for 26 weeks. Bedaquiline 400 mg every day for two weeks, followed by 200 mg three times a week for 64 weeks, and linezolid 1200 mg orally every day for 26 weeks.[28] The dose of linezolid can be adjusted to 600 mg, 300 mg or completely halted if required due to any known adverse drug reaction. This combination of drugs is to be taken with food. This treatment regime can be extended beyond 26 weeks if required.[25]

2.5.9. Adverse effects

Peripheral neuropathy, musculoskeletal pain, transaminases increased, dyspepsia, acne, anemia, nausea, vomiting, headache, and decreased appetite.[25]

2.5.10. Precautions

Hepatic adverse effects in patients getting BPaL combination treatment regimen. Avoid consumption of alcohol and any other hepatotoxic agents, including herbal supplements. Monitor liver enzymes like AST, ALP, ALT, and bilirubin if there is evidence of liver dysfunction.[25]

Myelosuppression in patients getting a combination BPaL regimen This is a known adverse reaction of the drug linezolid which can cause anemia which could be life-threatening. However, hematological changes due to the drug regimen were reversible after it was discontinued, reduced, or halted. Complete blood count monitoring should be done before the initiation of a drug regimen as a baseline and every month thereafter. If myelosuppression worsens, consider lowering or discontinuing linezolid.[25,27]

Peripheral and optic neuropathy in patients getting the BPaL regimen, which is a known adverse reaction of linezolid during its long-term use, this adverse reaction is reversible and improves with reduction, discontinuation, or interruption of linezolid dosing. Patients on this regimen should have their visual functions closely monitored. Peripheral and optic neuropathy in patients getting the BPaL regimen, which is a known adverse reaction of linezolid during its long-term use, this adverse reaction is reversible and improves with reduction, discontinuation, or interruption of linezolid dosing. Patients on this regimen should have their visual functions closely monitored. If experiencing visual impairment, halt linezolid dosing and get an ophthalmologic opinion.[25]

QT interval prolongation in BPaL combination regimen patients was a known adverse drug reaction to bedaquiline. ECG monitoring should be done before and 2, 12, and 24 weeks after the initiation of the treatment regimen. Serum electrolytes like potassium, calcium, and magnesium should be monitored at baseline and require specific treatment if found abnormal. In patients having a history of cardiac conditions like torsade de pointes, bradyarrhythmia, and heart failure, initiation of bedaquiline should be considered after risk-benefit analysis and ECG monitoring. If a patient precipitates a clinically significant QTc interval greater than 500 ms or ventricular arrhythmia, the treatment regimen must be halted.[25] Reproductive effects such as testicular atrophy and impaired fertility in male rats' Clinical reproductive toxicity studies have not been assessed.

Lactic acidosis in patients on the BPaL combination regimen, a known adverse reaction of linezolid. Patients on this drug regimen developing nausea or vomiting must be immediately evaluated for serum bicarbonate and lactic acid levels. If abnormal, the entire regimen or linezolid must be halted.[25,28]

2.6. Various clinical trial's data

Study 1–Double-blind placebo-controlled with two escalating-dose (single-dose and multiple-dose study) clinical studies were conducted to evaluate pharmacokinetics, safety and tolerability of PA-824. PA-824 showed good tolerability with minimal serious adverse events in total fifty eight healthy volunteers. Pharmacokinetic results of both studies showed that maximum plasma levels of PA-824 were achieved in 4–5 hours independent of the dose with average Cmax. of 3 μg/ml and 3.8 μg/ml in single-dose study (1,500 mg) and multiple-dose study (600 mg), respectively. Pa-824 required 5–6 days of daily dosing to achieve the steady state concentration with average elimination half-life of 16–20 hours. Overall study result showed good tolerability and consistent pharmacokinetic parameters with single dose of PA-824 once daily for up to 7 days.[23]

Study 2–Subjects with newly diagnosed TB, untreated TB, and sputum smear positive TB were included in the dose ranging randomized study to evaluate pharmacokinetics, tolerability, safety and early bactericidal activity of PA-824. Patients were divided into dose groups of 50 mg, 100 mg, 150 mg, and 200 mg once daily. Sputum samples were collected for two nights before drug administration, daily from the 1st to 4th day, and after that on alternate days till day 14. Once daily standard treatment regimen HRZE was given as positive control to 8 subjects. Mean rate of decline in Log CFU of *Mycobacterium tuberculosis* in a sputum smear incubated from overnight sputum collection was defined as primary way to measure efficacy of the drug expressed as Log10 CFU/day/ml sputum (± standard deviation). PA-824 was well tolerated. Only two serious adverse drug events were reported, one of which led to premature withdrawal from the study. Incidence of AE varied from 67% in 50 mg dosage group to 33% in 100 mg and 150 mg dosage groups. Similar percentage of (13–20%) AEs in each PA-824 groups were considered drug related. QT prolongation was seen in all groups in no patient it exceeded 480 ms, >60 ms increase from baseline was observed in one patient.[29]

Study 3–A multicenter, partially random, open label, phase 2b trial was conducted that recruited sputum smear positive for acid-fast bacilli patients of above 18 years of age having resistance against rifampin or drug sensitive for MTB from seven different sites of south Africa, 1 of Uganda, 2 of Tanzania for a prospective study. Susceptible group patients were susceptible to rifampicin and isoniazid whereas those included in drug resistant group were having resistance against rifampicin but were sensitive towards fluoroquinolones. GeneXpert [Cepheid, Sunnyvale, CA, USA], a molecular level assay method was used for spotting MTB for its different sensitivity patterns. Patients were further tested for fluoroquinolone resistance by MTBDR*sl* [Hain lifescience]. Drug-susceptible tuberculosis subjects were randomly assigned in a ratio of 1:1:1 for 56 days of treatment with either a standard regime of HRZE (oral isoniazid, rifampicin, pyrazinamide, and ethambutol), or pretomanid (oral 200 mg daily) and pyrazinamide (oral 1500 mg daily) with oral bedaquiline 400 mg daily from day 1–14 followed by 200 mg dose for three days in a week ($B_{load}PaZ$) or bedaquiline 200 mg daily ($B_{200}PaZ$). Rifampicin-resistant TB patients received $B_{200}PaZ$ regimen along with moxifloxacin 400 mg daily for 56 days. The effectiveness of the study was determined based on daily variations in the time taken percentage (TTP) of sputum smears that were positive. Study finding showed highest daily percentage change in TTP (5.17%) was exhibited by $B_{200}PaZ$ regimen followed by $B_{load}PaZ$ (4.87%) and HRZE group (4.04%).[15] Significant difference in bactericidal activity was observed in B200PaZ and $B_{load}PaZ$ arm compared to HRZE arm. A higher incidence of adverse events was reported in the $B_{load}PaZ$ (10%) and $B_{200}PaZ$ (8%) group in contrary to HRZE group (3%) leading to discontinuation of the study drug. A major reoccurring high-grade adverse event was elevated liver enzymes, which resulted in the withdrawal of 10 individuals (5 of the $B_{load}PaZ$ regime, 3 in the $B_{200}PaZ$ regime, and 2 in the HRZE regime). Two patients of the $B_{load}PaZ$ regime (3%) and one of the HRZE group (2%) experienced treatment-related serious adverse events (SAE). Study

Table 2
Adverse effects.

Study	Adverse effects
Study 1	Headache, elevated serum creatinine levels, stomach discomfort (nausea, vomiting, flatulence, and/or diarrhea), and back pain
Study 2	Pneumothorax and pneumonia, neither of them was considered drug-related
Study 3	Liver enzyme elevations
Study 4	Hepatotoxicity, delayed ventricular repolarization (i.e., QTc-prolongation), which can lead to torsade de pointes and fatal arrhythmias

results showed that because of their bactericidal action, both regimens have potential to reduce treatment duration and thus have promising effect in treatment of drug-susceptible TB patients.[30]

Study 4–In this study data from phase 3 and 2 clinical trials were evaluated in which QTc concentration remodeling method was applied to pretomanid. Other than pretomanid alone various drug combinations with bedaquiline, linezolid, moxifloxacin and pyrazinamide are available. BPaL regimen which has shown efficacy in Nix-TB study in subjects with XDR-TB or treatment intolerant or non-responsive MDR-TB got special attention. An increasing trend of QTc was seen with an increase in drug plasma concentration of bedaquiline metabolite, pretomanid, and moxifloxacin in a manner described by a linear model. The intercept increased from treatment to plateau after several weeks, obtaining a persistent pattern. For a dose of 200 mg OD every day, the typical maximum steady-state concentration of pretomanid in plasma resulted in a change of 9.1 ms QTc interval from baseline with an upper limit of 10.2 ms. In BPaL regimen additional impact of bedaquiline metabolite on values were 13.6 ms and 15.0 ms, by which the contribution of bedaquiline metabolite comes out to be 4.0 ms from the secular trend.[21] Anti-tubercular drugs such as fluroquinolones are also responsible for QTc interval prolongation by inhibition of voltage-gated potassium channels mostly delayed rectifier potassium current component. Among all floroquinolones greatest risk of QT interval is of moxifloxacin. Floroquinolones such as levofloxacin, Gemifloxacin, and ofloxacin are also associated with low-risk QTc interval prolongation. Ciprofloxacin is linked with the lowest risk (see Table 2).[31]

3. Discussion & conclusion

The persistent spread of MDR-TB has become one of the most crucial and burdensome challenges in order to restrain global TB.[32] Poor health-care systems, rising resistance due to ineffective treatment, and continued spread in health-care facilities and communities have emerged as the leading causes of MDR-TB strain transmission.[12] This article provides a comprehensive review of the drug "pretomanid," which has been approved by the US-FDA for use in patients infected with resistant MTB species/strains. Pretomanid has shown promising results and appears as safe, effective, and well tolerated in clinical studies. Headache is most commonly reported adverse events. High dose administration of this drug has caused an elevation in serum creatinine levels.[33] In vitro studies of this drug claim that maximum blood levels of PA-824 concentration were 6–200 folds higher than the minimum inhibitory concentration of MTB, both drug-susceptible and resistant species. Two major adverse drug events noticed were spontaneous pneumothorax at the 50 mg dosage group and pneumonia developed at the 200 mg dosage group but neither adverse drug event was drug-related. Moreover, this study shows QT prolongation in patients of all groups but did not exceed 480 ms $>$ 60 ms of increase from baseline was seen in patients of group 50–100 mg and 30 ms of increase from baseline was seen at 150 mg–200 mg dose.[29]

Authors' note

All authors have contributed equally toward the preparation of this case report. All authors read and approved the final manuscript. Written informed consent was obtained from the patient for publication of this case report.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Acknowledgment

The authors express their sincere gratitude to medical staff of MMIMSR Hospital, Maharishi Markandeshwar (Deemed to be University), Mullana (Ambala) for their support and provision of information.

References

1. MacNeil A, Glaziou P, Sismanidis C, Date A, Maloney S, Floyd K. Global epidemiology of tuberculosis and progress toward meeting global targets - worldwide, 2018. *MMWR Morb Mortal Wkly Rep*. 2020;69(11):281–285. https://doi.org/10.15585/mmwr.mm6911a2.
2. From WHO. Multidrug-Resistant Tuberculosis (MDR-TB). https://www.who.int/news-room/questions-and-answers/item/tuberculosis-multidrug-resistant-tuberculosis (MRD-TB). Published 2022. [Accessed June 19, 2022].
3. From Centers for Disease Control and Prevention https://www.cdc.gov/tb/topic/basics/signsandsymptoms.htm. Published 2022. [Accessed June 21, 2022].
4. From WHO. Extensively Drug-Resistant Tuberculosis (XDR-TB). https://www.who.int/news-room/questions-and-answers/item/tuberculosis-extensively-drug-resistant-tuberculosis-(XDR-TB) Published 2022. [Accessed June 21, 2022].
5. Jilani TN, Avula A, Zafar Gondal A, Siddiqui AH. Active Tuberculosis. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2023. https://www.ncbi.nlm.nih.gov/books/NBK513246/.
6. Naeem A, Rai SN, Pierre L. Histology, alveolar macrophages. In: *StatPearls. Treasure Island*. (FL: StatPearls Publishing; 2023. https://www.ncbi.nlm.nih.gov/books/NBK513313/.
7. Heemskerk D, Caws M, Marais B, Farrar J. *Tuberculosis in Adults and Children*. London: Springer; 2015. https://www.ncbi.nlm.nih.gov/books/NBK344402.
8. Tuberculosis 2: Pathophysiology and Microbiology of Pulmonary Tuberculosis. Southsudanmedicaljournal.com. http://www.southsudanmedicaljournal.com/archive/feb-2013/tuberculosis-2-pathophysiology-and-microbiology-of-pulmonary-tuberculosis.html.Published 2022. [Accessed June 21, 2022].
9. Narasimhan P, Wood J, Macintyre CR, Mathai D. Risk factors for tuberculosis. *Pulm Med*. 2013;2013, 828939. https://doi.org/10.1155/2013/828939.
10. Nahid P, Dorman SE, Alipanah N, et al. Official American thoracic society/centers for disease control and prevention/infectious diseases society of America clinical practice guidelines: treatment of drug-susceptible tuberculosis. *Clin Infect Dis*. 2016;63(7):e147–e195. https://doi.org/10.1093/cid/ciw376.
11. Dartois VA, Rubin EJ. Anti-tuberculosis treatment strategies and drug development: challenges and Priorities. *Nat Rev Microbiol*. 2022;27:1–17. https://doi.org/10.1038/s41579-022-00731-yeah.
12. Seung KJ, Keshavjee S, Rich ML. Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. *Cold Spring Harb Perspect Med*. 2015;5(9), a017863. https://doi.org/10.1101/cshperspect.a017863.
13. Salari N, Kanjoori AH, Hosseinian-Far A, Hasheminezhad R, Mansouri K, Mohammadi M. Global prevalence of drug-resistant tuberculosis: a systematic review and meta-analysis. *Infect Dis Poverty*. 2023;12(1):57. https://doi.org/10.1186/s40249-023-01107-x.
14. Diriba G, Alemu A, Yenew B, et al. Epidemiology of extensively drug-resistant tuberculosis among patients with multidrug-resistant tuberculosis: a systematic review and meta-analysis. *Int J Infect Dis*. 2023;132:50–63. https://doi.org/10.1016/j.ijid.2023.04.392.
15. Mase SR, Chorba T. Treatment of drug-resistant tuberculosis. *Clin Chest Med*. 2019;40(4):775–795. https://doi.org/10.1016/j.ccm.2019.08.002.
16. From WHO. *Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*. vol. 5. Geneva: World Health Organization; 2014. Treatment strategies for MDR-TB and XDR-TB https://www.ncbi.nlm.nih.gov/books/NBK247431/.
17. Vanino E, Granozzi B, Akkerman OW, et al. Update of drug-resistant tuberculosis treatment guidelines: a turning point. *Int J Infect Dis*. 2023;130(suppl 1):S12–S15. https://doi.org/10.1016/j.ijid.2023.03.013.
18. Diarra B, Toloba Y, Konate B, et al. Extensively drug resistant tuberculosis in Mali: a case report. *BMC Res Notes*. 2017;10(1):561. https://doi.org/10.1186/s13104-017-2890-4.

19. Keam SJ. Pretomanid: first approval. *Drugs*. 2019;79(16):1797–1803. https://doi.org/10.1007/s40265-019-01207-9.
20. Singh R, Manjunatha U, Boshoff HI, et al. PA-824 kills nonreplicating Mycobacterium tuberculosis by intracellular NO release. *Science*. 2008;322(5906):1392–1395. https://doi.org/10.1126/science.1164571.
21. Stover CK, Warrener P, VanDevanter DR, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature*. 2000;405(6789):962–966. https://doi.org/10.1038/35016103.
22. Baptista R, Fazakerley DM, Beckmann M, Baillie L, Mur LAJ. Untargeted metabolomics reveals a new mode of action of pretomanid (PA-824). *Sci Rep*. 2018;8(1):5084. https://doi.org/10.1038/s41598-018-23110-1.
23. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects. *Antimicrob Agents Chemother*. 2009;53(9):3720–3725. https://doi.org/10.1128/AAC.00106-09.
24. Lyons MA. Modeling and simulation of pretomanid pharmacokinetics in pulmonary tuberculosis patients. *Antimicrob Agents Chemother*. 2018;62(7), e02359-17. https://doi.org/10.1128/AAC.02359-17.
25. Gomez GB, Siapka M, Conradie F, et al. Cost-effectiveness of bedaquiline, pretomanid and linezolid for treatment of extensively drug-resistant tuberculosis in South Africa, Georgia and the Philippines. *BMJ Open*. 2021;11(12), e051521. https://doi.org/10.1136/bmjopen-2021-051521.
26. Yang JS, Kim KJ, Choi H, Lee SH. Delamanid, bedaquiline, and linezolid minimum inhibitory concentration distributions and resistance-related gene mutations in multidrug-resistant and extensively drug-resistant tuberculosis in Korea. *Ann Lab Med*. 2018;38(6):563–568. https://doi.org/10.3343/alm.2018.38.6.563.
27. Shuman M, Lee Demler T, Trigoboff E, Opler LA. Hematologic impact of antibiotic administration on patients taking clozapine. *Innov Clin Neurosci*. 2012;9(11–12):18–30.
28. Mao Y, Dai D, Jin H, Wang Y. The risk factors of linezolid-induced lactic acidosis: a case report and review. *Medicine (Baltim)*. 2018;97(36), e12114. https://doi.org/10.1097/MD.0000000000012114.
29. Diacon AH, Dawson R, du Bois J, et al. Phase II dose-ranging trial of the early bactericidal activity of PA-824. *Antimicrob Agents Chemother*. 2012;56(6):3027–3031. https://doi.org/10.1128/AAC.06125-11.
30. Tweed CD, Dawson R, Burger DA, et al. Bedaquiline, moxifloxacin, pretomanid, and pyrazinamide during the first 8 weeks of treatment of patients with drug-susceptible or drug-resistant pulmonary tuberculosis: a multicentre, open-label, partially randomized, phase 2b trial. *Lancet Respir Med*. 2019;7(12):1048–1058. https://doi.org/10.1016/S2213-2600(19)30366-2.
31. Briasoulis A, Agarwal V, Pierce WJ. QT prolongation and torsade de pointes induced by fluoroquinolones: infrequent side effects from commonly used medications. *Cardiology*. 2011;120(2):103–110. https://doi.org/10.1159/000334441.
32. Prasad R, Gupta N, Banka A. Multidrug-resistant tuberculosis/rifampicin-resistant tuberculosis: principles of management. *Lung India*. 2018;35(1):78–81. https://doi.org/10.4103/lungindia.lungindia_98_17.
33. Conradie F, Diacon AH, Ngubane N, et al. Treatment of highly drug-resistant pulmonary tuberculosis. *N Engl J Med*. 2020;382(10):893–902. https://doi.org/10.1056/NEJMoa1901814.

Indian Journal of Tuberculosis 71 (2024) S136–S140

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Study of adverse drug reactions during the treatment of drug resistant tuberculosis

Diya Dutta Gupta [1,*], Sanjivani J. Keny, Uday C. Kakodkar

Department of Respiratory Medicine, Goa Medical College, Bambolim, Goa, 403202, India

A R T I C L E I N F O

Keywords:
Multidrug resistant tuberculosis
Adverse drug reactions
Pharmacovigilance
Prevention and control
Drug toxicity
Adverse drug event
Kochs disease
Infection
Mycobacterium tuberculosis
Drug-resistant tuberculosis
Drug related side effects and adverse reaction

A B S T R A C T

Background: Pharmacovigilance entails monitoring of patients for timely detection of ADR and reporting them so that more information about drug safety can be obtained. This may help in the future for dose modification or alteration of regimen. In NTEP, ADSm (Active Drug Safety monitoring) is part of pharmacovigilance. In this study we shall be studying ADRs to Anti TB drugs in DRTB.
Methodology: This study is observational, retrospective and record based, of patients admitted from 2021 to 2023 in the DOTS ward of Respiratory Medicine Department of a tertiary care hospital in Goa. Data such as age, sex, regimen, date of AKT initiation and adverse effects documented has been noted and compiled.
Results: ADRs have been tabulated in the form of tables.
Statistical analysis is done to find out the commonest ADR, time when they are likely to occur, which age and gender are most likely affected and if there are any other associated risk factors for ADRs.
Conclusion: This study will enable in future to better monitor patients with regard to particular adverse drug reaction, patient safety and if needed to alter the regimen as early as possible.

1. Introduction

Pharmacovigilance entails monitoring of patients for timely detection of ADR and reporting them so that more information about drug safety can be obtained. This may help in the future for dose modification or alteration of regimen.[1]

In NTEP, ADSm (Active Drug Safety monitoring) is part of pharmacovigilance. In this study we have studied ADRs to Anti TB drugs in DRTB[2]

Since the introduction of newer anti TB drugs such as delamanid and bedaquiline, and newer regimens in India through the Programmatic Management of Drug Resistant Tuberculosis Guidelines in 2021,[2] there has been a pressing need for more studies outlining their adverse effects and tolerability. Timely detection and intervention for ADR is necessary to improve patient compliance and ensure the MDR TB is effectively treated.

In our study we have documented all the adverse effects encountered in patients admitted in our DOTS plus ward since adopting the new guidelines in 2021.

2. Aims and objectives

The aim is to study the adverse drug reactions during treatment of drug resistant tuberculosis. The objective of the study is to evaluate for adverse drug reactions during the course of treatment for MDR TB.

3. Materials and methods

The study design is observational, retrospective and record based. It is conducted in the DOTS plus ward of the Respiratory Medicine Department of a tertiary care hospital in Goa. The study population consists of the patients with drug resistant tuberculosis admitted in the DOTS plus ward and the study sample consists of patients with drug resistant tuberculosis having adverse reactions to the Anti-TB treatment received. The type of sampling used is the census sampling method.

4. Eligibility

All patients admitted in the DOTS plus ward between 2021, 2022 and 2023 with multidrug resistant tuberculosis and receiving treatment for

* Corresponding author. Department of Respiratory Medicine, Goa Medical College, Bambolim, Goa, 403202, India.
E-mail address: drddg13@gmail.com (D. Dutta Gupta).
[1] Permanent address- Flat C 402, Springfield Apartments, Near Bank of India Taleigao branch, 403002. Goa, India. Phone no- 7798346437

https://doi.org/10.1016/j.ijtb.2024.03.002
Received 12 January 2024; Received in revised form 28 February 2024; Accepted 5 March 2024
Available online 6 March 2024

drug resistant tuberculosis were included in this study and patients below the age of 12 years were excluded.

5. Data collection and statistical analysis

Approval of the Institutional Ethics Committee was obtained prior to commencing the study.

Data collection was done retrospectively from records of patients admitted in the DOTS plus ward (Directly Observed Treatment Short Course) from 2021 to June of 2023. Statistical analysis has been done to find out the commonest adverse event, time when they are likely to occur, which age and gender are most likely affected, and the commonest drug likely to be causing the adverse drug reactions.

As per the latest PMDT 2021 guidelines, once a patient is diagnosed as a case of Rifampicin resistant tuberculosis, one of two regimens may be initiated; the all-oral shorter regimen consisting of bedaquiline, levofloxacin, clofazimine, pyrazinamide, ethambutol, isoniazid and ethionamide. The duration of this regimen is 9–11 months. Bedaquiline is given for 6 months. The intensive phase lasts for 4–6 months and the continuation phase for 5 months. Only levofloxacin, clofazimine, pyrazinamide and ethambutol are continued in the continuation phase. The second regimen is the all-oral longer regimen consisting of bedaquiline, levofloxacin, linezolid, clofazimine and cycloserine. The duration of this regimen is 18–20 months without a separate intensive and continuation phase. Bedaquiline is omitted after 6 months or treatment and linezolid is stepped down to 300 mg after 6 months.

In both the regimens, the drug dosage is as per the weight band. In the event that a particular drug needs to be replaced, the guidelines outline a replacement sequence for the same.

6. Results

The total number of patients found to be admitted with adverse drug reactions to multidrug resistant tuberculosis treatment in this study was found to be 48 as seen in (Fig. 1).

The average age of patients experiencing ADRs was found to be 38.7 years.

Of all those affected, 29 were male and 19 were female (Fig. 2).

The various regimens which caused drug reactions were enumerated (Fig. 3), and it was seen that the all-oral longer regimen caused ADR in 26 individuals, all oral shorter regimen caused ADR in 6 patients, few patients who were still completing ongoing older DR TB regimens

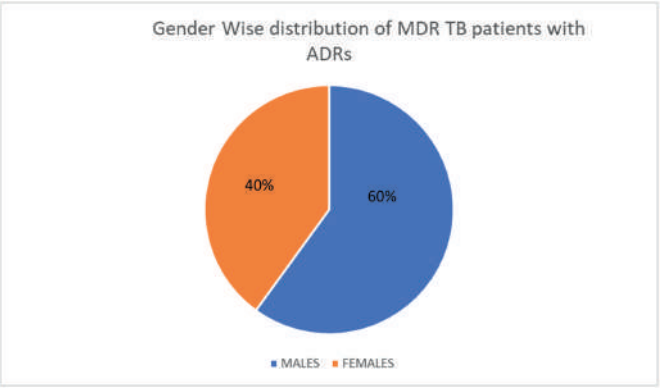


Fig. 2. Gender Wise distribution of MDR TB patients with ADRs.

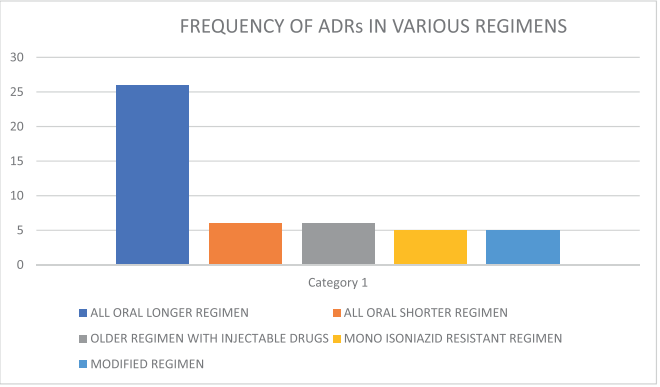


Fig. 3. Frequency of ADRs in various regiments

containing injectable drug caused ADR in 6 individuals, and mono isoniazid resistant regimen caused ADR in 5 individuals. Some patients were on modified regimen due to SLLPA showing resistance to any drug, of these 5 individuals suffered ADR.

The most common adverse drug reaction was found to be AKT induced gastritis in this study, with a total of 21 patients or 43.7% patients experiencing this adverse effect (Fig. 1). The second commonest ADR was found to be bone marrow suppression in the form of reduced

SERIAL NO.	ADVERSE DRUG REACTION [ADR]	FREQUENCY	SUSPECTED DRUG CAUSING ADR
1	AKT induced gastritis	21	Non-specific, AKT induced
2	Bone marrow suppression	10	Linezolid
3	QTc prolongation	9	Bedaquiline, Clofazimine
4	Psychiatric disturbances	8	Cycloserine
5	Peripheral neuropathy	4	Linezolid
6	Itching	2	Non-specific
7	Nephrotoxicity	2	Kanamycin, Cycloserine
8	Other ECG changes [T wave inversions, ectopics]	2	Non-specific
9	Palpitations	2	Non-specific
10	Arthralgia	1	Pyrazinamide, FQ
11	Sensorineural hearing loss	1	Kanamycin
12	Angioedema	1	Levofloxacin
13	Seizure	1	Levofloxacin
14	Injection site painful induration	1	Amikacin
15	Temporal field defect	1	Linezolid
16	Skin hyperpigmentation	1	Clofazimine

Fig. 1. Frequency of adverse drug reactions
ADR- Adverse Drug reactions, AKT-anti tubercular therapy, BDQ-bedaquiline, FQ-fluoroquinolones.

hemoglobin and reduced total red and white blood cell and platelet counts compared to baseline seen in 20% of patients. The third most common ADR was seen to be QTc prolongation seen in 18% of the patients. And 4th and 5th commonest ADR was found to be psychiatric disturbances (seen in 16% of patients) followed by peripheral neuropathy (seen in 8% of patients).

44% of the males and 42% of females experienced AKT induced gastritis in this study (Fig. 4). QTc prolongation was found to be present in a much higher frequency in females in this study, with 36% females versus only 10% males having prolonged QTc after starting the regimen. Cycloserine induced psychiatric disturbances were seen in a higher percentage of females (21%) as compared to males (13%) Bone marrow suppression was seen in 21% females as compared to 20% males in this study.

Among the drugs administered, non-specific AKT induced gastritis was the most common adverse drug reaction. Among individual drugs, Linezolid was associated with the highest number of Adverse drug reactions (15), Followed by Bedaquiline (10) and Cycloserine (9).

In this study in was found that within the first week, the most common ADR encountered were AKT induced gastritis, followed by QTc prolongation. In the first 3 months of starting AKT, bone marrow suppression, psychiatric adverse effects and nephrotoxicity were most likely to manifest. And beyond 3 months ADRs such as peripheral neuropathy and rarely bone marrow suppression was encountered.

In those with AKT induced gastritis, the symptoms were treated with H-2 blockers and patient education about the symptoms and to take the tablets with food. In case of severe gastritis AKT was withheld for a few days, symptomatic treatment given, and restarted once symptoms subsided. All patients with severe gastritis eventually restarted treatment with these measures.

In all the cases of linezolid induced bone marrow suppression, linezolid was either omitted or replaced by drugs according to the sequence of replacement drugs enumerated in the PMDT 2021 guidelines. 3 patients required packed red cell blood transfusion. All patients underwent a full recovery after omission or replacement of linezolid.

Linezolid was omitted in all cases of peripheral neuropathy or replaced with other drugs. In all cases the symptoms subsided but some amount of paraesthesia persisted.

In the cases of QTc prolongation, the AKT was withheld, electrolyte imbalances were ruled out, cardiology department clearance was obtained, and in all patients, we were able to restart AKT by reintroducing the drugs one at a time with QTc monitoring except in one patient who had QTc more than 500 despite normal electrolytes. Bedaquiline was omitted in that particular case and since that patient also had linezolid induced bone marrow suppression as well as peripheral neuropathy, the final regimen at discharge consisted of pyrazinamide, ethionamide, ethambutol, cycloserine and amikacin.

Cycloserine induced psychiatric disturbances were treated by omitting cycloserine or replacing it. Patients experienced visual and auditory hallucinations, delusions, aggression towards others and developed self-harming behavior. After psychiatric evaluation and treatment all patients recovered from their symptoms completely.

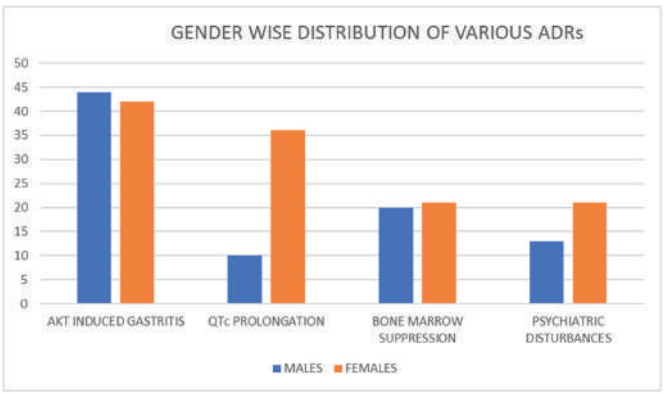


Fig. 4. Gender wise distribution of various ADRs.

Other less frequent side effects included nephrotoxicity (induced by kanamycin and cycloserine), sensorineural hearing loss (kanamycin), angioedema and seizure induced by levofloxacin, temporal field defect caused by linezolid, painful injection site induration (amikacin). For all the these ADRs the offending drugs were omitted or replaced.

There were also cases of itching, palpitation, arthralgia, skin hyperpigmentation (clofazimine). In these cases, the symptoms subsided with supportive treatment and did not warrant change in regimen.

7. Discussion

Adverse drug reaction is defined by the WHO as "an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product."[3]

The importance of early recognition and timely intervention for ADRs in MDR TB patients is that for a successful cure and to limit the spread of drug resistant strains, treatment adherence is paramount. In a systematic review, it was found that in India, side effects of drugs were one of the leading causes leading to default of treatment.[4]

In this study the sample size was limited, having 48 patients who were admitted in the DOTs ward with adverse drug reactions from 2021 to 2023. This can be attributed to the relatively low population of this state.

The average age was found to be 38.7 years, which is similar to the study conducted by Dela AI et al., and other studies[5]

The majority of the sample was found to be male (60%), which is in accordance with other studies done in 2022 in Odisha[6] and Telangana.[7]

The most common adverse effect in this study was AKT induced gastritis followed by anemia and QTc prolongation, as compared to the study by Paikray E, Das P, Pattnaik M et al., which showed QTc prolongation to be the most commonly encountered side effect.[6]

Gastrointestinal upset (nausea, vomiting, gastritis) was found to be one of the most debilitating ADR leading to treatment interruption in a retrospective cohort study[8,9] as well as the most common in a study by Massud A et al.[10] hence should be promptly recognized and treated. In our study patients improved with symptomatic treatment and with H2 blockers therapy. In severe cases the drugs were withheld for a few days and then reintroduced with careful patient education and modifications such as taking the tablets after eating some food.

Linezolid induced ADRs were the most common in this study with bone marrow suppression being more frequent than peripheral neuropathy. This is not in accordance with a retrospective multicenter study done in India in 2023 regarding the adverse drug reactions of linezolid in the programmatic management of DR TB, which showed peripheral neuropathy to be the most commonly encountered ADR.[11] According to Zou, Fan, Zhiwei Cui et al.[12] who did a study purely on the adverse effects of linezolid, the commonest adverse effect is thrombocytopenia followed by anemia.

Only one case of linezolid induced temporal field defect was seen in this study, which is a rare linezolid ADR as seen in a study by Aljebreen MA et al..[13] Although 6 of the patients in this study with ADR to linezolid had diabetes mellitus type 2 which is a confounding factor for peripheral neuropathy, the dominant side effect was still bone marrow suppression as opposed to peripheral neuropathy.

Linezolid induced peripheral neuropathy can oftentimes be very painful and debilitating[14] and often even after omission of the drug there may be lasting sensory symptoms which may cause severe distress to patients[15,16] and hence must be identified and treated as early as possible.

Bedaquiline is well documented to have caused QTc prolongation in multiple studies[8,17,18] In this study bedaquiline was seen to cause

prolonged QTc in 18% of the patients, of which 30% were males and 70% were female. QTc values are considered normal when they are between 350 and 450 ms for males and between 360 and 460 ms for females.[19] Apart from bedaquiline, other drugs which are known to have potential QTc prolonging effects are delamanid, clofazimine and fluroquinolones.[20,21] In all cases except one in this study, bedaquiline was withheld and reintroduced after electrolyte imbalances were corrected and with prior cardiology consultation. It was omitted in only one patient. Clinically significant QTc prolongation and cardiotoxicity enough to warrant withdrawal of the drug and change in regimen is rare as seen in a study conducted by Obaid et al. and Guglielmetti et al.[22,23]

Cycloserine was found to be the third commonest drug causing ADRs in this study. Cycloserine has a higher frequency of causing psychiatric and central nervous system adverse drug reactions (such as psychosis, aggressive behavior, suicidal ideation) compared to other drugs used in DR TB regimen.[24,25] Psychiatric disturbances in our patients included aggressive, violent behavior towards self and others, visual and auditory hallucinations and delusions as seen in a study by Yadav et al.[26,27] With prompt omission of cycloserine and counselling sessions and psychiatric medical treatment, all patients recovered and their symptoms subsided completely, similar to another study by Cotrina et al.[16] However, cycloserine induced psychosis may also lead to suicide as seen in a study done by Behera et al.[28] hence physicians must be vigilant and aware of this adverse effect.

A few infrequent adverse drug reactions noted in this study include 2 cases each of kanamycin and cycloserine induced nephrotoxicity, kanamycin induced sensorineural hearing loss, levofloxacin induced angioedema and seizure in 2 separate individuals and painful injection site induration caused by amikacin. In all these cases the offending drugs were omitted or replaced with other drugs from the replacement sequence and symptoms were treated with a multidisciplinary approach. These side effects are similarly documented in other studies.[29–34]

In this study, maximum number of adverse effects occurred within one week of starting AKT and included AKT induced gastritis followed by QTc prolongation. Hence close monitoring is recommended in the first week of starting AKT. In the first 3 months of starting AKT, bone marrow suppression, psychiatric adverse effects and nephrotoxicity were most likely to occur. Routine monitoring of complete blood counts is recommended, along with vigilance for psychiatric adverse effects. And beyond 3 months ADRs such as peripheral neuropathy was more commonly encountered with a few cases of bone marrow suppression as well and the findings are similar to those seen in other studies[35,36]

This study describes and documents the ADRs encountered in a tertiary care center in Goa in MDR TB patients and needs to be compared with similar studies in different locations and time spans to validate the findings and aid in management strategies.

8. Conclusion

Treatment adherence and cure is dependent upon early recognition and prompt treatment of ADRs. In this study the commonest ADR was AKT induced gastritis, followed by anemia and QTc prolongation. Most of the adverse effects occurred within one week of starting the treatment. It is thus important to carefully educate the patients about the various risks of the regimens at initiation, and to ensure surveillance. More studies are required to improve the programmatic management of DR TB and ADRs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. WHO REFERENCE NUMBER: WHO/HTM/TB/2015.28. Active Tuberculosis Drug-Safety Monitoring and Management (aDSM): Framework for Implementation.
2. *Guidelines for Programmatic Management of Drug Resistant Tuberculosis in India.* 2021.
3. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. *Lancet*. 2000 Oct 7;356(9237):1255–1259. https://doi.org/10.1016/S0140-6736(00)02799-9. PMID: 11072960.
4. Tola HH, Tol A, Shojaeizadeh D, Garmaroudi G. Tuberculosis treatment non-adherence and lost to follow up among TB patients with or without HIV in developing countries: a systematic review. *Iran J Public Health*. 2015 Jan;44(1):1–11. PMID: 26060770; PMCID: PMC4449995.
5. Dela AI, Tank NKD, Singh AP, Piparva KG. Adverse drug reactions and treatment outcome analysis of DOTS-plus therapy of MDR-TB patients at district tuberculosis centre: a four year retrospective study. *Lung India*. 2017 Nov-Dec;34(6):522–526. https://doi.org/10.4103/0970-2113.217569. PMID: 29098997; PMCID: PMC5684809.[.
6. Paikray E, Das P, Pattnaik M, et al. Adverse drug reaction monitoring in multidrug-resistant tuberculosis patients receiving bedaquiline and delamanid-based regimen. October 27 *Cureus*. 2022;14(10), e30764. https://doi.org/10.7759/cureus.30764.
7. Fatima S, Syeda MF, Adla N, Devi R. Ambispective study of adverse drug reactions in multi-drug resistant tuberculosis patients in Warangal, Telangana. *Lung India*. 2021 Jul-Aug;38(4):330–337. https://doi.org/10.4103/lungindia.lungindia_118_19. PMID: 34259171; PMCID: PMC8272421.
8. Gupta A, Kumar V, Natarajan S, Singla R. Adverse drug reactions & drug interactions in MDR-TB patients. *Indian J Tubercul*. 2020. https://doi.org/10.1016/j.ijtb.2020.09.027.
9. Russom M, Verhamme K, Karimi L, Ahmed H. Nature, Magnitude and risk factors of adverse drug reactions in multidrug resistant tb patients in Eritrea. *Eur Respir J [Internet]*. 2018;52(suppl 62) [cited 2020 Jul 22] https://erj.ersjournals.
10. Massud A, Syed Sulaiman SA, Ahmad N, Shafqat M, Chiau Ming L, Khan AH. Frequency and management of adverse drug reactions among drug-resistant tuberculosis patients: analysis from a prospective study. *Front Pharmacol*. 2022 Jun 2;13, 883483. https://doi.org/10.3389/fphar.2022.883483. PMID: 35747749; PMCID: PMC9211428.
11. Mishra Gyanshankar, Alffenaar Jan-Willem, Munje Radha, Khateeb Sadaf. Adverse drug reactions due to linezolid in the programmatic management of drug-resistant tuberculosis in India: a retrospective multicenter study. *Indian J Tubercul*. 2023. https://doi.org/10.1016/j.ijtb.2023.04.006. ISSN 0019-5707.
12. Zou Fan, Cui Zhiwei, Lou Siyu, et al. Adverse drug events associated with linezolid administration: a real-world pharmacovigilance study from 2004 to 2023 using the faers database. *Front Pharmacol*. 2024;15. https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2024.1338902.
13. Aljebreen MA, Alotaibi AK, Alrobaian M. Linezolid-induced toxic optic neuropathy. *Middle East Afr J Ophthalmol*. 2021 Jan 19;27(4):235–237. https://doi.org/10.4103/meajo.MEAJO_73_20. PMID: 33814822; PMCID: PMC7993054.
14. Tambe Rituja, Doshi Preeti P. Management of linezolid-induced peripheral neuropathy. *Indian Journal of Pain*. 2023;37(2):120–122. https://doi.org/10.4103/ijpn.ijpn_115_22. May–Aug.
15. Lifan Z, Sainan B, Feng S, Siyan Z, Xiaoqing L. Linezolid for the treatment of extensively drug-resistant tuberculosis: a systematic review and meta-analysis. *Int J Tubercul Lung Dis*. 2019;23:1293–1307.
16. Rho JP, Sia IG, Crum BA, Dekutoski MB, Trousdale RT. Linezolid-associated peripheral neuropathy. *Mayo Clin Proc*. 2004;79:927–930.
17. Diacon AH, Pym A, Grobusch MP, et al. Multidrug-resistant tuberculosis and culture conversion with bedaquiline. *N Engl J Med*. 2014;371:723–732. https://doi.org/10.1056/NEJMoa1313865.
18. Darmayani IGAAPS, Ascobat P, Instiaty I, Sugiri YJR, Sawitri N. Bedaquiline effect on QT interval of drugs-resistant tuberculosis patients: real world data. *Acta Med Indones*. 2022 Jul;54(3):389–396. PMID: 36156479.
19. Postema P, Wilde A. "The measurement of the QT interval. *Curr Cardiol Rev*. 2014; 10:287–294.
20. Choudhri Shurjeel H, Harris Louise, Butany Jagdish W, Keystone Jay S. Clofazimine induced cardiotoxicity–a case report. *Lepr Rev*. 1995;66(no. 1):63–68.
21. Amankwa Kwadwo, Krishnan Subramaniam C, Tisdale James E. Torsades de pointes associated with fluoroquinolones: importance of concomitant risk factors. *Clin Pharmacol Therapeut*. 2004;75(3):242–247.
22. Obaid Ur Rehman, Fatima Eeshal, Ali Abraish, Akram Umar, Nashwan Abdulqadir, Yunus Faryal. Efficacy and safety of bedaquiline containing regimens in patients of drug-resistant tuberculosis: an updated systematic review and meta-analysis. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*. 2024;34, 100405. https://doi.org/10.1016/j.jctube.2023.100405. ISSN 2405-5794.
23. Guglielmetti Lorenzo, Tiberi Simon, Burman Matthew, et al. QT prolongation and cardiac toxicity of new tuberculosis drugs in Europe: a Tuberculosis Network European Trialsgroup (TBnet) study. *Eur Respir J*. 2018;52(2).
24. Cotrina-Santome A, Ulloa-Esquivel L, Vásquez-Quispe S, et al. Cycloserine-induced psychosis in patients with drug-resistant tuberculosis: a systematic review of case reports. *Egypt J Neurol Psychiatry Neurosurg*. 2023;59:37. https://doi.org/10.1186/s41983-023-00642-6.
25. Dissmann E. "Experience with cycloserine." scandinavian journal of respiratory diseases. *Supplement*. 1970;71:239–243.
26. Yadav S. Cycloserine-induced insomnia and psychosis in multidrug-resistant pulmonary tuberculosis - a case report. *Cureus*. 2022 Dec 26;14(12), e32963. https://doi.org/10.7759/cureus.32963. PMID: 36712725; PMCID: PMC9875866.

27. Yadav Sankalp, Rawal Gautam. Adverse drug reactions due to cycloserine on the central nervous system in the multidrug-resistant tuberculosis cases: a case series. *PAMJ-Clinical Medicine*. 2019;1:25.
28. Behera C, Krishna Karthik, Singh HR. Antitubercular drug-induced violent suicide of a hospitalised patient. *Case Reports*. 2014;2014, bcr2013201469.
29. Ghafari N, Court R, Chirehwa MT, et al. Pharmacokinetics and other risk factors for kanamycin-induced hearing loss in patients with multi-drug resistant tuberculosis. *Int J Audiol*. 2020 Mar;59(3):219–223. https://doi.org/10.1080/14992027.2019.1690170. Epub 2019 Nov 18. PMID: 31739701; PMCID: PMC7582222.
30. Perumal R, Abdelghani N, Naidu N, et al. Risk of nephrotoxicity in patients with drug-resistant tuberculosis treated with kanamycin/capreomycin with or without concomitant use of tenofovir-containing antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2018 Aug 15;78(5):536–542. https://doi.org/10.1097/QAI.0000000000001705. PMID: 29683992.
31. Wang J, Pang Y, Jing W, et al. Efficacy and safety of cycloserine-containing regimens in the treatment of multidrug-resistant tuberculosis: a nationwide retrospective cohort study in China. *Infect Drug Resist*. 2019 Apr 3;12:763–770. https://doi.org/10.2147/IDR.S194484. PMID: 31040707; PMCID: PMC6452793.
32. Sheth Haiya J, Shikha V Sood, Patel Rushin P, Malhotra Supriya D. A case report on angioedema induced by levofloxacin: an unexpected occurrence. *Int J Basic Clin Pharmacol*. 2017;6(11):2749–2751. https://doi.org/10.18203/2319-2003.ijbcp20174800.
33. Espinosa-Pereiro J, Sánchez-Montalvá A, Aznar ML, Espiau M. MDR tuberculosis treatment. *Medicina*. 2022 Jan 26;58(2):188. https://doi.org/10.3390/medicina58020188. PMID: 35208510; PMCID: PMC8878254.
34. Garcia-Prats AJ, Rose PC, Draper HR, et al. Effect of coadministration of lidocaine on the pain and pharmacokinetics of intramuscular amikacin in children with multidrug-resistant tuberculosis: a randomized crossover trial. *Pediatr Infect Dis J*. 2018 Dec;37(12):1199–1203. https://doi.org/10.1097/INF.0000000000001983. PMID: 29561515; PMCID: PMC6138584.
35. Törün T, Güngör G, Özmen I, et al. Side effects associated with the treatment of multidrug-resistant tuberculosis. *Int J Tubercul Lung Dis*. 2005;9(12):1373–1377.
36. Patel Sangita Vashrambhai, Bhikhubhai Nimavat Kapil, Patel Alpesh Bhimabhai, Samrat Shringarpure Kalpita, Mehta Kedar Gautambhai, Krishnakumar Shukla Lipy. Adverse drug reactions in patients put on multi drug resistant tuberculosis (MDR-TB) treatment in seven districts of Central Gujarat. *J Young Pharm*. 2015;7(4s):425–431.

Indian Journal of Tuberculosis 71 (2024) S141–S144

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Beyond the norm: Primary multidrug-resistant extrapulmonary tuberculosis unveiled in a case series

Manju Bala [a], Akhlesh Rajpoot [a,*], Madhur Kalia [b]

[a] Department of Respiratory Medicine, Soban Singh Jeena Government Institute of Medical Science and Research, Almora, Uttarakhand, India
[b] ENT Surgeon, Department of Head and Neck Surgery, District Hospital, Mohali, Punjab, India

ARTICLE INFO

Keywords:
EPTB
MDR-TB
Tubercular otitis media
Skeletal TB

ABSTRACT

Tuberculosis is a preventable and generally curable infectious disease caused by *Mycobacterium tuberculosis*. It mostly affects the lungs causing pulmonary tuberculosis; however, it may also involve non-pulmonary organs resulting in extrapulmonary tuberculosis (EPTB). Diagnosis of tuberculosis was based on the constitutional symptoms, organ-specific radiographs, and biological specimen examination. However, diagnosis of extrapulmonary tuberculosis can be difficult when the lungs are not affected and constitutional signs and symptoms of tuberculosis that can help to identify the disease are absent. Although multi-drug-resistant extrapulmonary tuberculosis is not uncommon, primary drug-resistant extrapulmonary tuberculosis in certain areas such as the extraspinal osteoarticular joint, tympanic membrane, and central nervous system is still rare. In this piece, we present three cases of primary multidrug-resistant extrapulmonary tuberculosis with an unusual presentation.

1. Introduction

Tuberculosis (TB) is a communicable disease caused by *Mycobacterium tuberculosis* (MTB), which is an acid-fast bacillus. It is a significant public health issue with preventable mortality and morbidity worldwide, especially in low-income countries like India.[1] The most common way to contract TB is through inhalation of aerosols containing MTB, which primarily affects the lungs resulting in pulmonary tuberculosis (PTB), which accounts for about 80% of all TB cases.[2] Extrapulmonary tuberculosis (EPTB) affects the extrapulmonary organs and occurs secondary to hematogenous, lymphatic, or contagious spread from the underlying focus of tuberculosis. Extrapulmonary TB commonly affects the lymph nodes, pleura, gastrointestinal tract, and central nervous system (CNS).[3] Tuberculosis of the ankle joint and otitis media are rare, accounting for less than 1% of cases, therefore, vigilance is necessary for timely diagnosis and proper treatment of these conditions. The situation becomes more complicated and challenging with the increased numbers of drug-resistant tuberculosis (DRTB) cases, particularly in low-income and high-burden TB countries.[4] This task becomes even more challenging when primary multidrug-resistant extrapulmonary tuberculosis (MDR-EPTB; with no prior history of TB or MDR-TB contact) lacks typical clinical features, and these uncommon presentations can be easily misdiagnosed, leading to a delay in treatment initiation. Here, we report three cases of primary MDR-EPTB in immunocompetent individuals.

Case presentation:

Case 1. Primary multidrug-resistant tuberculosis of right ankle joint.

A male patient 45 years of age presented with painful swelling in his right ankle for the past four years. The patient denied any history of fever, cough, night sweats, loss of appetite, or weight loss, and had no known pre-existing comorbidity. All routine blood tests, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RA), and anti-cyclic citrullinated peptide (CCP) were unremarkable. Upon examination, the right ankle displayed signs of inflammation, tenderness, and fluctuating swelling (Fig. 1a). However, the radiograph of the right ankle and chest showed no abnormalities (Fig. 1a and b respectively). A fine needle aspiration from the right ankle swelling was negative for bacterial and fungal infection. Still, it revealed rifampicin and isoniazid-resistant MTB, on Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) and Line Probe Assay (LPA).

Case 2. Primary multidrug-resistant tuberculosis of the right middle ear.

A 26-year-old woman presented with a complaint of purulent discharge and reduced hearing from her right ear for one month. She

* Corresponding author.
E-mail address: akhileshrajpoot855@gmail.com (A. Rajpoot).

https://doi.org/10.1016/j.ijtb.2024.03.010
Received 29 January 2024; Received in revised form 8 March 2024; Accepted 22 March 2024
Available online 23 March 2024

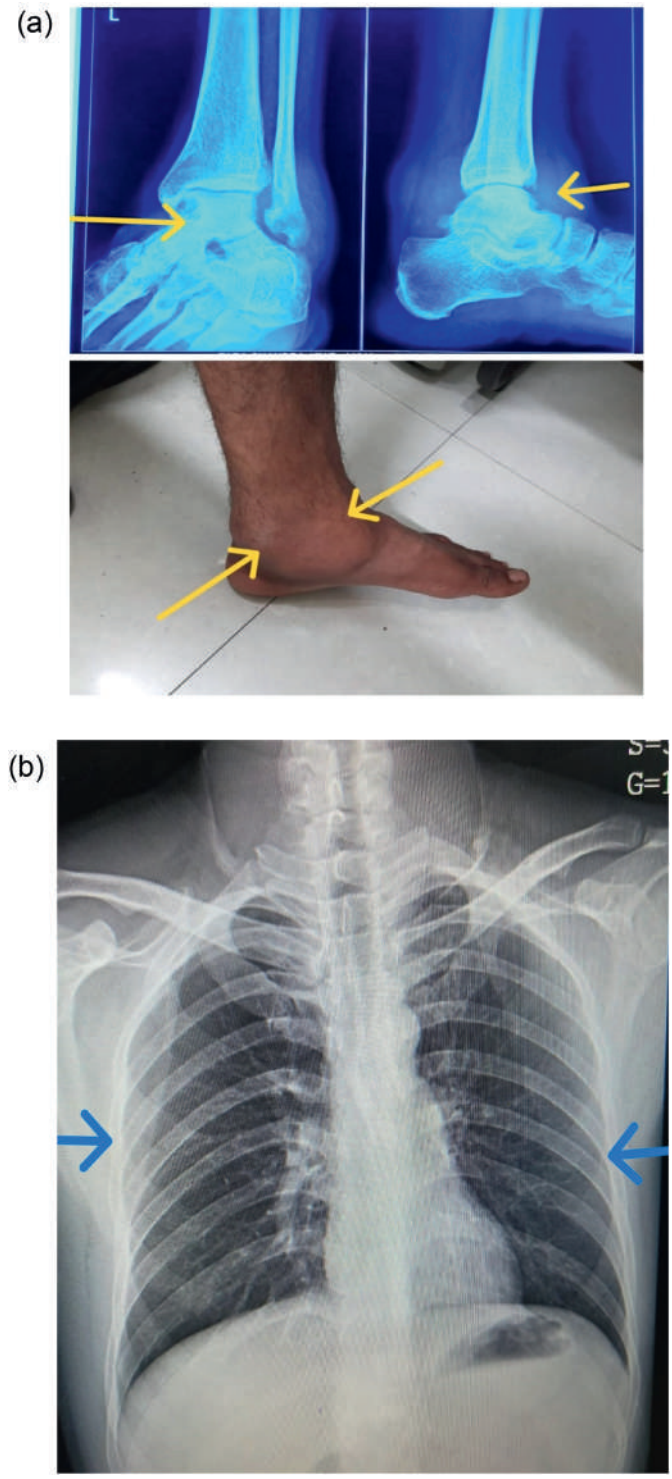


Fig. 1. (a) : Arrowheads showing a normal radiograph of the right ankle with, inflammation, and swelling of the right ankle.
(b): Arrowheads showing a normal chest radiograph.

denied experiencing fever, cough, sore throat, weight loss, or other constitutional symptoms of TB and she had no known underlying co-morbidity. Despite receiving systemic and local antibiotics, the ear discharge did not improve. Upon examination, the perforated tympanic membrane with purulent discharge was identified in the right ear (Fig. 2a); however, there were no other notable findings on systemic examination. Routine blood tests and chest radiographs were unremarkable (Fig. 2b). A swab was taken from the right ear for CBNAAT and

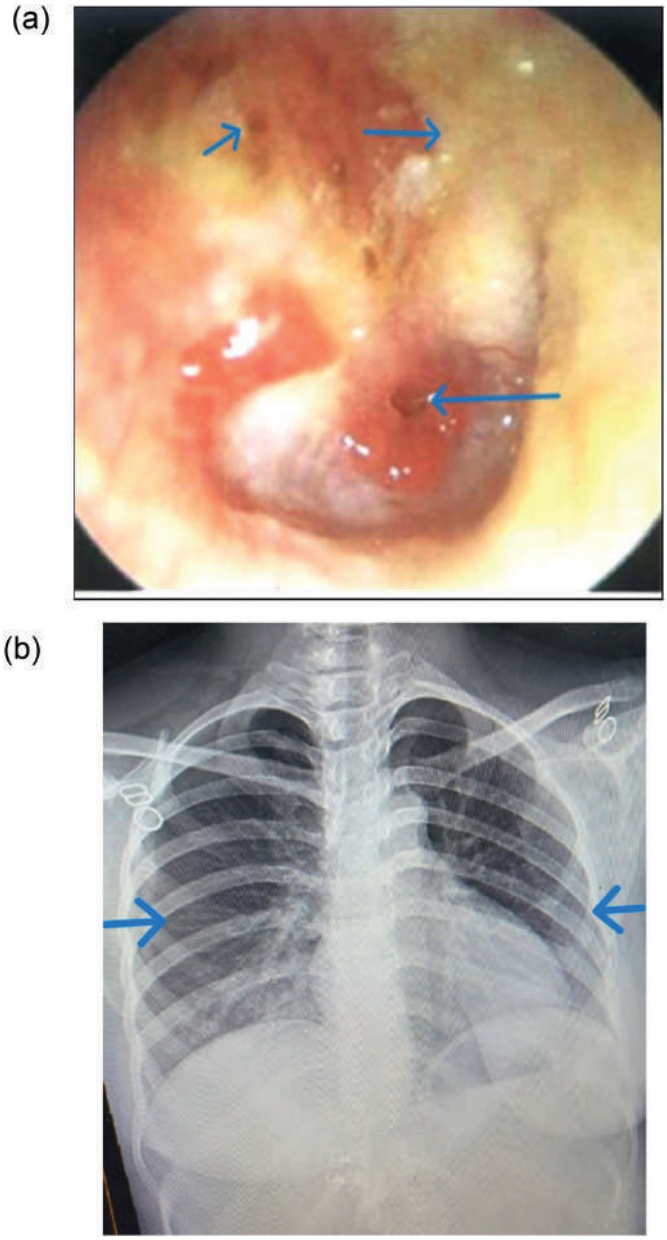


Fig. 2. (a) : Arrowheads showing right tympanic membrane perforation and purulent discharge.
(b): Arrowheads showing a normal chest radiograph.

LPA testing, which confirmed a diagnosis of primary MDR-EPTB.

Case 3. Primary multidrug-resistant tuberculosis (disseminated) of the left middle ear and brain.

A 38-year-old man has been experiencing headaches for three months and has noticed a decrease in hearing in his left ear for the past two months. He denied experiencing cough, fever, sore throat, or any other constitutional symptoms of TB, and any other pre-existing co-morbidity. A left ear exam revealed a perforated tympanic membrane with purulent discharge, and a pus swab was taken. However, the rest systemic examination revealed no abnormalities. All routine blood tests and chest radiographs were normal (Fig. 3a). A non-contrast computed tomography (NCCT) of the head was performed, which revealed a hypodense lesion in the right frontal region (Fig. 3b), and a lumbar puncture was performed for cerebrospinal fluid (CSF) analysis. The pus swab from the left ear and CSF analysis were unremarkable for bacterial

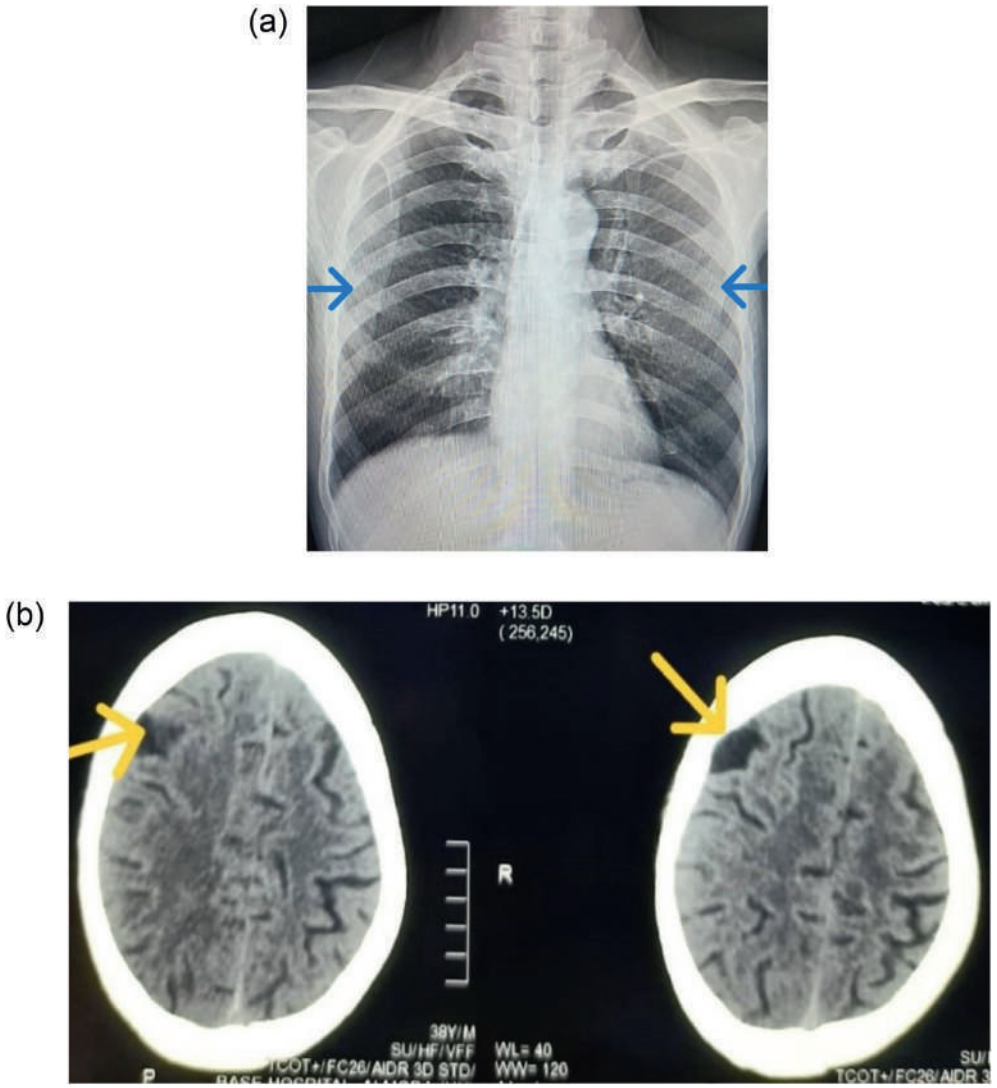


Fig. 3. (a): Arrowheads showing a normal chest radiograph
(b): Arrowheads showing hypodense lesion in the right frontal region.

and fungal infection, however, MDR-TB was detected on CBNAAT and LPA testing.

Following a comprehensive clinical examination and laboratory investigations, all three patients received treatment for MDR-TB with a combination of five drugs (Levofloxacin, Bedaquiline, Linezolid, Clofazimine, and Cycloserine), under the PMDT-2021 program. They showed clinical improvement, tolerated the treatment well, and completed it in 18 months.

2. Discussion

The article is about the rare cases of primary MDR-EPTB that affect different body parts other than the lungs. Although PTB is the more prevalent form of the disease, a smaller proportion of patients (approximately 15–20%) may develop EPTB.[5] Extrapulmonary TB can affect almost any living organ in the human body and is difficult to recognize. Lymph node TB constitutes almost half of all EPTB cases, followed by pleura, genitourinary, skeleton, gastrointestinal, and CNS.[6] Around 10.6 million people are affected by TB worldwide and almost half a million of them have MDR-TB. India has the highest number of MDR-TB cases, accounting for 27% of all MDR-TB cases.[1] Currently WHO categorizes drug-resistant TB into 5 types: isoniazid-resistant TB, rifampicin-resistant TB, MDR-TB, pre-extensively drug-resistant TB (pre-XDR-TB), and XDR-TB.[7] There are different ways of detecting drug resistance and sensitivity in TB. These include genotypic methods, such as CBNAAT/TruNAAT, LPA, and whole genome sequencing, and phenotypic methods, such as liquid and solid media culture.

Skeletal TB is seen in about 9% of cases of EPTB, however, ankle joint tuberculosis is rare, especially primary MDR-TB of the ankle.[6] Tubercular osteomyelitis of the involved joint is common and present in about 98% of cases of skeletal tuberculosis but in our case, only soft tissue of the ankle joint is involved, which is also uncommon.[8] Constitutional symptoms like fever, night sweats, appetite, and weight loss are generally not present in EPTB, which makes it difficult to diagnose and leads to delays in diagnosis. Tuberculosis of the tympanic membrane is uncommon and it accounts for a small proportion of all cases of chronic otitis media, about 0.05–0.9%.[9] Tubercular otitis media is generally a sequela of pulmonary, pharynx, larynx, or nose infection with TB, which can occur through hematogenous spread, aspiration via eustachian tube, and direct implantation of MTB through perforated tympanic membrane or external auditory meatus.[10] Tubercular otitis media remains a diagnostic dilemma due to its non-specific presentation. Classical signs and symptoms of tubercular otitis media like painless otorrhea and multiple tympanic membrane perforation are inconsistent features. Tympanic membrane findings may be varied, and it can have single or multiple perforations. Our one patient had primary disseminated MDR-EPTB (ear

and brain), which is a rare presentation of tuberculosis.

Primary MDR-EPTB of the ankle joint, brain, and middle ear is extremely rare, and CNS MDR-TB cases only constitute 0.12% of all DRTB cases and 2.6% of extrapulmonary MDR-TB.[11] Patients with tubercular otitis media usually have PTB, so they must be screened for PTB by chest radiography and sputum examination. However, some patients with tubercular otitis media, CNS, and skeletal TB do not develop PTB, and their symptoms and signs are similar to other chronic diseases, resulting in diagnostic delays. Radiological imaging is important in diagnosing and assessing the severity of TB in the CNS, skeletal system, and middle ear. However, biological specimens are necessary to confirm the diagnosis of TB and to evaluate the patterns of drug resistance. Once the diagnosis of DRTB is confirmed, patients must be treated with appropriate anti-tubercular treatment (ATT) for a sufficient duration, following the PMDT guidelines to ensure effective treatment. Sometimes, surgical intervention may be required in addition to ATT for bone erosion in skeletal TB or tubercular otitis media with poor drainage or severe CNS tuberculosis.

Recent studies reported the treatment success rate of the MDR-TB regimen ranges from 50 to 75%.[12,13] However, the BPaLM regimen significantly improves success rates for treating MDR-TB, with reported success rates of up to 89%.[14] It could be a promising treatment option for MDR-TB. All three patients were diagnosed with primary MDR-EPTB and managed with an extended oral regimen containing Bedaquiline (six months), Levofloxacin, Linezolid, Clofazimine, and Cycloserine, under the PMDT-2021 guidelines. After 18 months of treatment initiation, all the patients showed significant clinical progress and were declared to have completed their treatment successfully.

3. Conclusion

Multidrug-resistant tuberculosis affecting organs such as the ankle joint, brain, and middle ear is an infrequent condition that requires specialized doctors with a good understanding and knowledge of the disease. Unfortunately, doctors who specialize in treating these organs, such as orthopedists, neurologists, and otolaryngologists, may not have enough knowledge about DRTB, leading to delayed diagnosis due to the disease's rarity and non-specific clinical presentation. Therefore, it is important to consider TB as a possible cause of long-standing otitis media and osteoarticular swelling that doesn't respond to conservative treatment, especially in areas where TB is common. Rapid, compassionate, and specific diagnostic methods to detect DRTB should also be used for all suspected TB specimens.

Funding

Nil.

Availability of data and materials

The data used to support the findings of this report are available from the corresponding author upon request.

Patient consent for publication

Taken.

Prior publication

Nil.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We wish to thank all the patients for their well-being and for providing valuable consent.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijtb.2024.03.010.

References

1. World Health Organization. *Global Tuberculosis Report*; 2023. who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023 . Accessed November 14, 2023.
2. Bruzgielewicz A, Rzepakowska A, Osuch-Wojcikewicz E, et al. Tuberculosis of the head and neck-epidemiological and clinical presentation. *Arch Med Sci*. 2014;10(6):1160–1166. https://doi.org/10.5114/aoms.2013.34637.
3. Fanning A. Tuberculosis: 6 extrapulmonary disease. *CMAJ (Can Med Assoc J)*. 1999;160:1597–1603.
4. Shah HD, Nazli Khatib M, Syed ZQ, et al. Gaps and interventions across the diagnostic care cascade of TB patients at the level of patient, community and health system: a qualitative review of the literature. *Trav Med Infect Dis*. 2022;7:136. https://doi.org/10.3390/tropicalmed7070136.
5. Gambhir S, Ravina M, Rangan K, Dixit M, Barai S, Bomanji J. Imaging in extrapulmonary tuberculosis. *Int J Infect Dis*. 2017;56:237–247. https://doi.org/10.1016/j.ijid.2016.11.003.
6. Heye T, Stoijkovic M, Kauczor H, Junghanss T, Hosch W. Extrapulmonary tuberculosis: radiological imaging of an almost forgotten transformation artist. *Röfo*. 2011;183(11):1019–1029. https://doi.org/10.1055/s-0031-1273429.
7. WHO. Global tuberculosis report 2021. *Geneva: World Health Organization*; 2021. Licence: CC BY-NC-SA 3.0 IGO http://978-92-4-003702-1.
8. Kulchavenya E, Naber KG, Johansen TEB. *Epidemiology of extrapulmonary tuberculosis in sener A and Erdem H. Extrapulmonary Tuberculosis*. Cham: Springer; 2019:1–14.
9. Djeric Dragoslava, Tomanovic Nada, Boricic Ivan. Tuberculous otitis media diagnosis and treatment of four cases. *J. Int. Adv. Otol.* 2013;9(2), 255e259. https://doi.org/10.1016/j.joto.2019.12.001.
10. Hoca NT, Ogretensory M, Dayioglu D. Tuberculous otitis media-a case report. *Turkish Resp. J*. 2008;9:3.
11. Desai U, Joshi JM. Extrapulmonary drug-resistant tuberculosis at a drug-resistant tuberculosis center, Mumbai: our experience – hope in the midst of despair. *Lung India*. 2019;36:3–7. https://doi.org/10.4103/lungindia.lungindia_192_18.
12. WHO. Consolidated guidelines on tuberculosis. Module 4: treatment- drug-resistant tuberculosis treatment. *Geneva: World Health Organization*; 2022. update https://iris.who.int/handle/10665/365308.
13. Alemu A, Bitew ZW, Worku T. Poor treatment outcome and its predictors among drug-resistant tuberculosis patients in Ethiopia, a systematic review and meta-analysis. *Int J Infect Dis*. 2020;98:420–439. https://doi.org/10.1016/j.ijid.2020.05.087.
14. Nyang'wa BT, Berry C, Kazounis E, et al. TB-PRACTECAL study collaborators. A 24-week, all-oral regimen for rifampin-resistant tuberculosis. *N Engl J Med*. 2022 Dec 22;387(25):2331–2343. https://doi.org/10.1056/NEJMoa2117166.

Indian Journal of Tuberculosis 71 (2024) S145–S148

Contents lists available at ScienceDirect

Indian Journal of Tuberculosis

journal homepage: www.journals.elsevier.com/indian-journal-of-tuberculosis/

Isoniazid resistance in Rifampicin sensitive pulmonary tuberculosis in children and adolescents

Anurag Agarwal [a], Priyanka Das [a,b], Surendra Bahadur Mathur [a,c,*], Mahmud Hanif [d], Kaushal Kumar Dwivedi [d], Ashwani Khanna [e], Reema Arora [e], Aashima Dabas [a]

[a] Department of Pediatrics, Maulana Azad Medical College & Associated Hospitals, Bahadur Shah Zafar Marg, New Delhi, 110002, India
[b] Department of Pediatrics, All India Institute of Medical Sciences, Bhubaneshwar, India
[c] Department of Pediatrics, Postgraduate Institute of Child Health, Sector-30, Noida, Gautam Buddha Nagar, 201303, India
[d] New Delhi Tuberculosis Centre, New Delhi, India
[e] Chest Clinic (TB), Lok Nayak Hospital, New Delhi, India

ARTICLE INFO

Keywords:
Drug resistance
Line probe assay
Mycobacterium growth indicator tube
Cartridge based nucleic acid amplification test
Antitubercular agents

ABSTRACT

Background: Isoniazid (INH) and Rifampicin (RIF) are two crucial drugs used in antitubercular therapy. INH is known for its potent bactericidal effects and has a relatively higher prevalence of resistance compared to RIF. However, RIF resistance has been the subject of more extensive research. On the other hand, Ethambutol (EMB) and Streptomycin (STR) resistance have not been thoroughly studied, particularly in the context of children and adolescents. To address this knowledge gap, a study was designed to investigate the resistance patterns of INH, EMB, and STR in RIF-sensitive pulmonary tuberculosis (PTB) cases among children and adolescents.
Methods: Seventy-five newly diagnosed RIF sensitive PTB cases up to 18 years of age were enrolled. Retreatment cases were excluded. Sputum/gastric aspirate sample of these patients were sent for culture in Mycobacterium Growth Indicator Tube (MGIT) followed by drug susceptibility testing and Line Probe Assay.
Results: INH, EMB and STR resistance among RIF sensitive PTB cases was found to be 5.7%, 0% and 0.7% respectively. RIF resistance detected by CBNAAT was found to be 8.4%.
Conclusion: Detection of INH resistance is as important as detecting RIF resistance as prevalence of INH resistance in RIF sensitive PTB among children and adolescents up to 18 years is around 6%.

1. Introduction

Tuberculosis (TB) is a communicable disease and one of the top 10 leading causes of death from a single infectious agent, ranking above HIV/AIDS and leads to around 1.5 million deaths annually.[1,2] India is estimated to have the highest burden of pediatric TB in the world and approximately 342 thousand children up to 14 years of age are estimated to get TB every year accounting for about 6–7% of all the patients treated under the National TB Elimination Program of India annually.[3,4]

Introduction of universal drug susceptibility testing (DST) in India emphasizes on testing all TB patients for resistance to at least Rifampicin (RIF) by cartridge based nucleic acid amplification test (CBNAAT).[5] Though guidelines suggest detection of Isoniazid (INH) resistance in all cases of RIF sensitive TB, according to the India TB Report 2021 and 2022, first line line probe assay (LPA) for detection of INH resistance has been performed only in 40% and 37.6% of the cases detected with Mycobacterium tuberculosis (MTb) by CBNAAT.[6,7]

First-line standardized regimen is preferably started after ruling out resistance to RIF upfront in all cases.[8] Patients with unknown INH resistance may be erroneously treated as drug sensitive cases, which may lead to treatment failure, relapse and emergence of multi drug resistant (MDR) TB. As drug resistant TB is becoming an emerging threat, detection of resistance to each and every drug has become an important cornerstone. Hence, this study was planned to find out the resistance of INH, Ethambutol (EMB) and Streptomycin (STR) in RIF sensitive PTB cases among children and adolescents.

* Corresponding author.
E-mail addresses: doctoranuragagarwal@gmail.com (A. Agarwal), peds.priyankadas.2020@gmail.com (P. Das), sbmathur05@gmail.com (S.B. Mathur), mahmudhanifkm@gmail.com (M. Hanif), dwivedikaushal@yahoo.com (K.K. Dwivedi), drashwani.khanna@gmail.com (A. Khanna), arorareema1569@gmail.com (R. Arora), dr.aashimagupta@gmail.com (A. Dabas).

https://doi.org/10.1016/j.ijtb.2024.05.002
Received 15 February 2024; Received in revised form 6 May 2024; Accepted 8 May 2024
Available online 9 May 2024

2. Materials & methods

This cross-sectional study was conducted in the department of pediatrics, chest clinic and associated TB centre of a tertiary teaching hospital from June 2021 to August 2022. The study was approved by the institutional ethics committee. A sample size of 136 was calculated at 95% confidence level with an absolute error of 5% and the percentage of INH resistance in newly diagnosed RIF sensitive pulmonary TB patients taken as 9.8% as per the study done by Tehseen et al.[9,10] However, for this study a sample size of convenience of 75 was taken. The study was registered in the Clinical Trial Registry - India.

This study was conducted among presumptive PTB patients up to 18 years of age attending outpatient and inpatient department of the hospital. A patient with presumptive PTB was defined as a patient having any of the following symptoms: persistent fever, cough for >2 weeks, loss of weight (>5% loss in the past 3 months) or failure to gain weight in the past 3 months despite adequate nutrition with no other apparent cause.[8] Sputum or gastric aspirate sample from all these patients was obtained and divided into 2 parts. One part of the sample was subjected to CBNAAT after sample processing according to standard technique for detection of MTb and RIF resistance.[11] Those who came positive for MTb and were not RIF resistant were enrolled for the study. Those cases who had already been started on antitubercular therapy (ATT) were excluded. Written informed consent and/or assent were obtained from all enrolled subjects. Detailed history taking and clinical examination was done in enrolled cases and second part of their sample was processed using standard technique for culture using Mycobacterium Growth Indicator Tube (MGIT) 960 automated system (Becton Dickinson, Sparks, MD, USA).[12]

Samples which showed growth in MGIT were subjected to phenotypic DST for INH, RIF, EMB and STR by the BACTEC MGIT 960 SIRE Kit for antimycobacterial susceptibility testing. The genotypic DST for INH and RIF was also done by LPA using GenoTypeMTBDRplus (Hain Lifescience, Nehren, Germany) as per manufacturer's instructions.

To maintain confidentiality of the patient, de-identified data were used for analysis. The collected data and DST results were entered, analyzed and statistically evaluated using IBM SPSS Statistics Version 25.0. Categorical data were presented as counts and percentages. Continuous data were expressed as mean and standard deviation.

3. Results

Sputum/Gastric aspirate sample of 455 suspected PTB patients were sent for CBNAAT. A flowchart depicting the study enrolment is shown in Fig. 1. Important baseline socio-demographic and clinical characteristics of the study participants have been mentioned in Table 1.

All the 75 samples were sent to the associated TB centre for culture by MGIT.Out of 75, 3 (4%) samples showed no growth in MGIT though

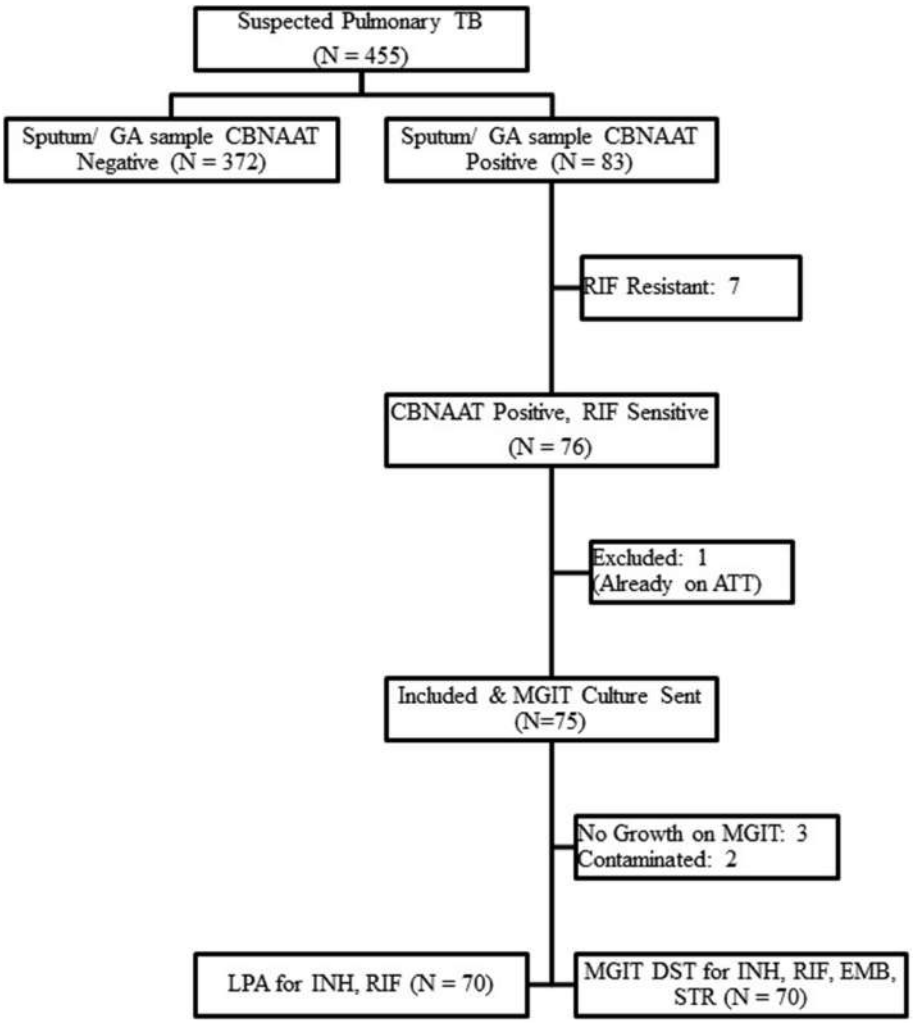


Fig. 1. Flowchart depicting study enrolment (TB: Tuberculosis; GA: Gastric Aspirate; CBNAAT: Cartridge Based Nucleic Acid Amplification Test; RIF: Rifampicin; ATT: Antitubercular therapy; MGIT: Mycobacterium Growth Indicator Tubes; LPA: Line Probe Assay; DST: Drug Sensitivity Testing; INH: Isoniazid; EMB: Ethambutol; STR: Streptomycin).

Table 1
Socio-demographic and Clinical characteristics of the study participants (N = 75).

Characteristic	Value
Female Gender	53 (70.7)
Age in years[a]	14.1 (3.74)
Age Group	
• Up to 12 years age	24 (32)
•12–18 years age	51 (68)
Weight for age <3rd centile	52 (69.3)
BMI for age/Weight for height <3rd centile	62 (82.7)
Fever at presentation	74 (98.7)
Duration of fever	
•< 2 weeks	1 (1.3)
•2–4 weeks	25 (33.3)
•> 4 weeks	48 (64)
Cough at presentation	71 (94.7)
Duration of cough	
•< 2 weeks	3 (4)
•2–4 weeks	27 (36)
•> 4 weeks	41 (54.7)
Chest Radiograph findings	
•Consolidation with hilar/paratracheal lymphadenopathy	38 (50.7)
•Only hilar/paratracheal lymphadenopathy	13 (17.3)
•Miliary pattern	11 (14.7)
•Only consolidation	5 (6.7)
•Cavitary lesion with lymphadenopathy	4 (5.3)
•Pleural effusion with lymphadenopathy	4 (5.3)
Mantoux Reactive	42 (56)
HIV Status	
•Negative	58 (77.3)
•Not Known	16 (21.3)
•Positive	1 (1.3)
Positive history of significant contact with a patient having tuberculosis	35 (46.7)

Data expressed as no. (%) or.
[a] mean (SD).

they had come MTb positive and Rifampicin sensitive by CBNAAT. Culture of 2 (2.7%) samples came contaminated. Seventy (93.3%) samples showed growth in MGIT and hence were subjected to phenotypic drug sensitivity test by MGIT SIRE system for STR, INH,RIF,EMB and first line LPA for INH and RIF. Four out of 70 samples i.e. 5.7% showed resistance to INH both in MGIT SIRE system and first line LPA. All the 4 samples showed the katG mutation. There was no discordance seen between phenotypic and genotypic drug sensitivity testings. One (0.7%) sample was resistant to STR by MGIT SIRE system and this STR resistance was not associated with any other drug resistance (INH, RIF, EMB). None of the samples were resistant to RIF either by MGIT SIRE system or LPA. Similarly, none of the samples showed resistance to EMB. There was concordance in the resistance status of RIF in all three modalities i.e. CBNAAT, MGIT and LPA.

Among the 4 INH resistant cases, 3 were female. All were more than 12 years of age with mean age of 17 years. All of them had fever. 3 out of 4 had fever for more than 4 weeks. 1 patient had fever for less than 2 weeks but he had cough for more than 4 weeks. All the INH resistant cases had cough for more than 4 weeks. Weight loss was present in all of them. Night sweat was present in only one case. 3 out of 4 cases had anorexia. History of significant contact with an open case of TB was present in only 1. This contact was a neighbor who had died more than 1 year back. We tried to find out more details but were unable to get any. One case was HIV positive, 1 was HIV negative and 2 cases were of unknown HIV status. Mantoux test was positive in all. On chest X-rays, all 4 cases had hilar/paratracheal lymphadenopathy, and 2 cases had consolidation.

4. Discussion

In this study conducted on RIF sensitive PTB patients aged up to 18 years at a tertiary hospital, we found INH resistance by MGIT SIRE and first line LPA in nearly 6% of the subjects. STR resistance was seen in only 0.7% of the subjects and none of the patients had EMB resistance. RIF resistance detected by CBNAAT was 8.4%.

In studies, done on adults, INH mono resistance was found to be 4–8.7% which is similar to our study.[13–19] In studies conducted in the pediatric population by Prajapati et al.(2009–2012) at Delhi, India and Tehseen et al. at Islamabad, Pakistan, INH mono resistance was found to be 3.1% and 11.9% respectively.[10,20] The study had shown higher resistance maybe as it was conducted at a referral centre catering to high risk and sicker patients. According to India TB report 2018, 2019, 2020, 2021 and 2022 INH mono resistance in India was 11%, 7.3% 5.87%, 6.8%, 6% respectively and in Delhi, it was 6%, 6.9% and 6% in the year 2019, 2020 and 2021 respectively.[3,5–7,21]The above INH resistance from India TB reports inlcude both newly diagnosed and retreatment cases including adult population with all types of TB. In our study, we have included only newly diagnosed PTB cases up to 18 years of age. Our study therefore suggests that INH resistance in pediatric population might be similar to that in adult population. However, since we took only newly diagnosed cases and the resistance in retreatment cases is higher, we suspect that INH resistance in newly diagnosed cases is increasing over the years.

RIF resistance in different studies was found to be 3.4–10.1%.[14–16,20] According to the India TB reports of 2019,2020,2021 and 2022 RIF resistance detected by CBNAAT in India was 8.5%, 7.5%, 10.2% and 9.8% respectively and in Delhi it was 9.5%, 9.5%, 9.8% and 9.8% respectively.[3,5–7] Similarly, RIF resistance detected by CBNAAT in pediatric population in Delhi was found to be 13%, 9.5%,10.7% and 11.2% in 2019, 2020, 2021 and 2022 respectively, which is similar to the results seen in our study.[3,5–7]

In studies including adults, STR mono resistance has been found to be 6.2%–10% which is higher than what is observed in our study.[13,15,17] This may be because those studies had included both new and retreatment cases. We could find only 2 studies where only newly diagnosed cases were included. These studies from Delhi, India done by Prajapati et al.(2009–2012) and Sharma et al.(2014–2016) found the STR mono-resistance to be 3.1% and 0% respectively.[16,20] We also obtained similar results.

Similarly, studies including adults conducted from 2009 to 2016 have found EMB mono-resistance to be 0%–1.5%.[13–17,20] We also obtained similar results. This suggests that there has not been any significant changes in EMB resistance in the last decade.

In comparison to drug sensitive TB, we couldn't find any specific demographic parameters, risk factors or clinical features in INH resistant TB. This suggests that detection of drug resistant TB on the basis of demographic parameters, risk factors or clinical features may be difficult. Hence, microbiological detection of drug resistance is essential.

As per our literature review, our study is the only study in India in the last decade which has evaluated INH resistance in RIF sensitive newly diagnosed PTB in children and adolescents up to 18 years. We had performed both LPA and MGIT culture of all the samples of the enrolled cases. Out of the 75 samples, 70 samples showed growth in MGIT culture which reflects the robust sample collection and processing methods used in our study. The study however had a few limitations which include a small sample size and lack of gene mutation analysis data of the INH resistant samples.

Our study found almost similar percentage of INH resistance in newly diagnosed RIF sensitive PTB cases among pediatric population as compared to studies in adult population including all types of TB irrespective of previous treatment status. Hence, in the current scenario of emerging INH resistance in RIF sensitive TB, detection of only RIF resistance is not sufficient enough for better disease control. Detection of INH resistance is equally important especially in pediatric age group in order to prevent treatment failure, relapse and emergence of MDR TB. MGIT followed by LPA should be done in all CBNAAT positive pediatric TB patients.

Consent to participate

Informed consent and/or assent from all parents and children, as applicable were obtained for inclusion in the study.

Funding/support

No funds, grants, or other support was received.

Details of IEC committee & approval

Maulana Azad Medical College and Associated Hospital Institutional Ethics Committee. IEC no: F.1/IEC/MAMC/(82/10/2020/No135). Dated 14/01/2021.

Trial registration

Clinical Trials Registry – India No. CTRI/2021/03/032203.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. *Global Tuberculosis Report 2019*. Geneva: World Health Organization; 2019. Available from: https://www.who.int/publications/m/item/global-tuberculosis-report-2019. Accessed August 22, 2022.
2. *Global Tuberculosis Report 2020*. Geneva: World Health Organisation; 2020. Available from: https://www.who.int/publications-detail-redirect/9789240013131. Accessed August 22, 2022.
3. India TB report, 2020. Available from:. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2020. https://tbcindia.gov.in/showfile.php?lid=3538. Accessed August 22, 2022.
4. Dodd PJ, Gardiner E, Coghlan R, Seddon JA. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. *Lancet Global Health*. 2014; 2(8):e453–e459.
5. India TB report. *Central TB Division*. New Delhi: Ministry of Health and Family Welfare; 2019, 2019. Available from: https://tbcindia.gov.in/WriteReadData/India%20TB%20Report%202019.pdf. Accessed August 22, 2022.
6. India TB report, 2021. Available from:. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2021. https://tbcindia.gov.in/showfile.php?lid=3587. Accessed August 22, 2022.
7. India TB report. *Central TB Division*. New Delhi: Ministry of Health and Family Welfare; 2022, 2022. Available from: https://tbcindia.gov.in/WriteReadData/IndiaTBReport2022/TBAnnaulReport2022.pdf. Accessed August 22, 2022.
8. Pediatric TB management guideline. *Central TB Division*. New Delhi: Ministry of Health and Family Welfare; 2022, 2022. Available from: https://tbcindia.gov.in/index1.php?lang=1&level=2&sublinkid=4200&lid=2848. Accessed August 10, 2023.
9. Cornfield J. Modern methods in the sampling of human populations. *Am J Public Health Nation's Health*. 1951;41:654–661.
10. Tahseen S, Khanzada FM, Rizvi AH, et al. Isoniazid resistance profile and associated levofloxacin and pyrazinamide resistance in rifampicin resistant and sensitive isolates/from pulmonary and extrapulmonary tuberculosis patients in Pakistan: a laboratory based surveillance study 2015-19. *PLoS One*. 2020;15(9), e0239328.
11. Sowjanya DS, Behera G, Ramana Reddy VV, Praveen JV. CBNAAT: a novel diagnostic tool for rapid and specific detection of mycobacterium tuberculosis in pulmonary samples. *Int J Health Res Modern Integr Med Sci (IJHRMIMS)*. 2014: 2394–8612. ISSN.
12. Bergmann JS, Fish G, Woods GL. Evaluation of the BBL MGIT (Mycobacterial growth indicator tube) AST SIRE system for antimycobacterial susceptibility testing of Mycobacterium tuberculosis to 4 primary antituberculous drugs. *Arch Pathol Lab Med*. 2000;124(1):82–86.
13. Fasih N, Rafiq Y, Jabeen K, Hasan R. High isoniazid resistance rates in rifampicin susceptible Mycobacterium tuberculosis pulmonary isolates from Pakistan. *PLoS One*. 2012;7(11), e50551.
14. Myneedu VP, Singhal R, Khayyam KU, et al. First and second line drug resistance among treatment naive pulmonary tuberculosis patients in a district under Revised National Tuberculosis Control Programme (RNTCP) in New Delhi. *J Epidemiol Glob Health*. 2015;5(4):365–373.
15. Bedewi Z, Mekonnen Y, Worku A, et al. Mycobacterium tuberculosis in central Ethiopia: drug sensitivity patterns and association with genotype. *New Microbes New Infect*. 2017;17:69–74.
16. Sharma SK, Chaubey J, Singh BK, Sharma R, Mittal A, Sharma A. Drug resistance patterns among extra-pulmonary tuberculosis cases in a tertiary care Centre in North India. *Int J Tubercul Lung Dis*. 2017;21(10):1112–1117.
17. Nega BH, Tafess K, Zewude A, Yenew B, Gilman SI, Ameni G. Spoligotyping and drug sensitivity of Mycobacterium tuberculosis isolated from pulmonary tuberculosis patients in the Arsi Zone of South Eastern Ethiopia. *New Microbes New Infect*. 2019;33, 100620.
18. Palani N, Premkumar M, Vaishnavee V, et al. Trends in rifampicin and isoniazid resistance in patients with presumptive TB. *Int J Tubercul Lung Dis*. 2022;26(5): 446–453.
19. Chandrasingh S, Nair S. Isoniazid resistance in patients with Extrapulmonary Tuberculosis using line probe assay in a private multispecialty hospital in Bangalore, India. *Indian J Med Microbiol*. 2022;40(2):303–305.
20. Prajapati S, Upadhyay K, Mukherjee A, et al. High prevalence of primary drug resistance in children with intrathoracic tuberculosis in India. *Paediatr Int Child Health*. 2016;36(3):214–218.
21. India TB report, 2022. Available from:. New Delhi: Central TB Division, Ministry of Health and Family Welfare; 2018. https://tbcindia.gov.in/showfile.php?lid=3314. Accessed August 22, 2022.

Guide for Authors

Before you begin

The *Indian Journal of Tuberculosis* (IJTB) is a quarterly journal published in January, April, July and October. It publishes original articles on tuberculosis, respiratory diseases, case reports, review articles, and abstracts of articles published in other medical journals and book reviews. Every issue contains editorial sections on contemporary subjects, radiology forum and a forum for readers to express their opinions on published articles and raise questions on subjects appearing in the journal. IJTB is indexed in Medline.

Manuscripts submitted to *Indian Journal of Tuberculosis* should not have been published previously or be under simultaneous consideration for publication by any other journal. Violation may lead to a retraction of the published article by the Journal and other actions as deemed necessary by the editor. All articles (including those invited) will be peer-reviewed, and accepted articles will be edited to the Journal's style. Accepted manuscripts become the permanent property of the Journal and may not be reproduced, in whole or in part, without the written permission of the editor.

Human and animal rights

Studies involving human subjects or animals should have received the approval of the institutional ethics committee. A statement to this effect and that informed consent was obtained from participating human subjects must be included in the manuscript text. Please ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (http://www.wma.net/en/30publications/10policies/b3/index.html); EU Directive 2010/63/EU for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm); Uniform Requirements for manuscripts submitted to Biomedical journals (http://www.icmje.org). The privacy rights of human subjects must always be observed.

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/ethicalguidelines.

Conflict of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. See also http://www.elsevier.com/conflictsofinterest. Further information and an example of a Conflict of Interest form can be found at: http://elsevier6.custhelp.com/app/answers/detail/a_id/286/p/7923/.

Submission declaration and Verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck, http://www.elsevier.com/editors/plagdetect.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted. Please give contribution of each author on the cover page of the manuscript.

Changes to authorship

Ideally there should not be any change in authorship after the manuscript is submitted. In situations where there has been an omission or substantial work is done when the article is revised, an author's name may be added. This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:

Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed upon by the editor.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Reporting Clinical Trials

All randomized controlled trials submitted for publication should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart. Please refer to the CONSORT statement website at http://www.consort-statement.org for more information. This

journal has adopted the proposal from the International Committee of Medical Journal Editors (ICMJE) which require, as a condition of consideration for publication of clinical trials, registration in a public trials registry. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. For this purpose, a clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration. Further information can be found at http://www.icmje.org.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright see http://www.elsevier.com/copyright). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated. Please see http://www.elsevier.com/funding.

Manuscript Submission

All new manuscripts will have to be submitted through *Indian Journal of Tuberculosis* online submission and review website (http://ees.elsevier.com/ijtb). Please follow the following steps to submit your manuscript:

1. Open the homepage of the Journal's website http://ees.elsevier.com/ijtb.
2. Register yourself for free by clicking on register on the top and create a user profile with a desired username and mandatory details. On submission of the information, you will receive an email confirming your registration along with the 'Password'.
3. Click "Log In" on the main navigation menu at the top of the journal screen to open the login page.
4. Enter your username and password in the appropriate fields (e-mailed to you at the time of registration).
5. Click "Author Log in", this takes you to the "Author Main Menu".
6. After that you can submit the manuscript by following the instructions provided on the screen.
7. Revised manuscripts can be uploaded online using the same log in.

If you have any problems in submission of your manuscript, please send us an email at injorheumat@gmail.com. Note : Please note that the username and password combination required for Elsevier Editorial System is different from your username and password combination used to "Track your paper" on the Elsevier "Authors' Home" website.

By submitting a manuscript online, the author agrees to the following:

8. The work is original and free from plagiarism.
9. It has neither been published, nor is it not under consideration for publication at another journal.
10. All authors are aware of the authorship order. The corresponding author shall be responsible in case of dispute.
11. Once published, copyright of manuscript shall stand transferred to the Journal.
12. 'Conflict of interest' if any, must be explicitly stated at the end of the manuscript.

Manuscripts must conform to the instructions given below:

General

Type the manuscript using 'Times New Roman' font, size 12 in double space throughout. Please arrange the manuscript as follows: Title page, Abstract, Introduction, Methods, Results, Discussion, and References. Number all pages consecutively, beginning with the title page. All figures and Tables must be referred to in the manuscript. Consult a recent issue of the Journal for details. Only the Title page should bear the names and addresses of the author(s). Editorials, viewpoint/ perspective and review articles are generally by invitation. However if you are interested in writing a review/ viewpoint, you can send an email to the editor with the topic and a short summary of contents to be included. The editor will convey his decision in 15 days' time.

Length of articles

Editorial text can be up to 1500 - 2000 words with references not exceeding 10.

Original articles deal with planned studies that have been duly completed and convey definite conclusions from the data presented in the text. Text can be up to 2500 words, a structured summary/ abstract of maximum 250 words, seven tables/figures and 35 references. Preliminary communications from research still in progress could be submitted exceptionally, if the topic is important and the interim results could be of interest.

Review articles are from those especially requested persons, who have acknowledged competence in given subjects. Text can be up to 4500 words, a structured or unstructured summary/ abstract of maximum 250 words, 10 tables/figures and 50 references. Leading articles are by those who have expertise in selected aspect of a subject.

Short communications can be of a text up to 1000 words, a summary/ abstract of 100 words, two tables/figures and 10 references.

Case reports present problems of unusual clinical interest which have been systematically and fully investigated and where a firm diagnosis has been established with reasonable certainty or the result of therapeutic management is of significance. Text can be up to 1000 words, a summary/ abstract of 100 words, two tables/figures and 10 references.

Workers in the field of **Tuberculosis and Respiratory Diseases** are invited to contribute to the **Radiology Forum** by submitting brief reports of patients with interesting clinical and radiological features for publication. These will be published, provided that:

(a) the condition is of clinical and radiological interest;
(b) photographs (10 cm × 8 cm) are of suitable quality for printing;
(c) the diagnosis in each case has been confirmed;
(d) the chest radiograph is accompanied by brief clinical account, not exceeding 500 words, and 5 references

Forum, in the form of letters to the Editor, provides a platform to readers for expressing their opinions and is a channel of communication with the journal and its readers. It could be used for making suggestions, scientific critique on published articles or for reaching independent conclusions, for asking questions on subjects covered by the journal and for providing supplementary information, either confirming or contradicting the conclusions reached in the article. Such letters can be up to a text of 1000 words with two tables/figures and 10 references. Only the most important agreements, disagreements/suggestions may be chosen for commenting. It is usual to send a copy of such letters to the authors for obtaining a response, if any, after editorial changes. The response, similarly, has to be brief and relevant.

Correspondence can be up to 500 words without tables or figures and 5 references.

Title page

In animal studies, the title should state the species; all other titles will refer to human studies. State names of authors (including first names), the departments and the institution where the work was done. Please do not add your academic qualifications, designation etc. State contribution of each author clearly. A short, running title, not exceeding 40 characters, should be provided. Please provide the name, postal address with PIN code, facsimile number and E-mail address of the author to whom communications and proofs are to be sent. Acknowledgements, if any, may be mentioned on this page.

Abstract / Summary

Original articles should include a structured abstract of upto 250 words under the following headings: Background/Objectives, Methods, Results, and Conclusions. [See Ann Intern Med 1990; 113: 69–76]. References should not be included. Upto 10 key words, not present in the title, to assist indexing, should be provided below the Abstract in alphabetical order; these may be obtained from the Medical Subject Headings (MeSH) database of National Library of Medicine, USA.

Acknowledgements

These should appear at the end of the manuscript and should be brief (not more than six lines). The source of funding as well as a disclosure statement mentioning conflict of interest, if any, should appear under this heading.

References

Number the references in the order in which they first appear in the text and identify the reference numbers in the text in superscript. References must be placed at the end of the manuscript. Please use recent references as much as possible. The responsibility for accuracy of references lies with the respective authors. The Journal is in agreement with the International Committee of Medical Journal Editors (http://www.icmje.org). The general arrangement, abbreviations of Journal names and punctuations followed are as per the Uniform Requirements for Manuscripts submitted to Biomedical Journals (http://www.icmje.org). Please pay attention to the style of references and punctuations as follows:

Journal article
List all authors when six or less as shown in the example below:
Tallon D, Chard J, Dieppe P. Exploring the priorities of patients with osteoarthritis of the knee. *Arthritis Care and Res* 2000; 13: 312–9.
When there are seven or more authors, list only the first six and add et al.

Book or monograph
Following is an example: Cassidy JT. Juvenile rheumatoid arthritis. In: *Textbook of Rheumatology* 6th ed, Kelly et al (eds) Philadelphia Saunders 2000; pp. 1297–313.

Tables

Each table should be typed on a separate page and numbered consecutively in Arabic numerals. Each table should have a title and all abbreviations should be explained in the footnote. Necessary explanatory notes, if any, may be given below the table.

Figures/Illustrations/Photographs

Photographs of 300 dpi or higher resolution may be submitted as 'jpeg', or 'tiff ' files in a zipped folder. In clinical photographs, identity of the subjects should be suitably masked; in case this is not possible, a written permission from the concerned person should accompany the manuscript.

Legends to Figures

The figure number (numbered consecutively in Arabic numerals), title and explanations of the figures should appear in the legend (not on the figure). Type the legends on a separate page. Enough information should be included to interpret the figure without reference to the text.

Units

All measurements must be in metric units, preferably with corresponding SI units in parentheses.

Editorial Process

All articles submitted to the Journal undergo initial review by the Editor and articles that are outside the scope of Journal or are not in the journal format are excluded. Later each article is reviewed by at least two reviewers. The time to first decision is usually less than 12 weeks.

As per the policy of the Journal, an Editor, who is either author of a manuscript or belongs to the same institution as any of the authors, is not assigned that manuscript and is not involved in decision-making regarding its publication. Reviewers/Editorial Board members should decline the invitation to review a manuscript which is submitted by authors from their institution. Editor is final authority on all decision making and reserves the right to make editorial corrections.

Correspondence

Address all correspondence related to the *Indian Journal of Tuberculosis* to: The Editor, *Indian Journal of Tuberculosis*, Tuberculosis Association of India, 3 Red Cross Road, New Delhi-110001.

Scopus®

Expertly curated abstract & citation database

ELSEVIER

Reaxys®

High quality and transparent data

ELSEVIER

Contents Continued from IBC