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Original Research Article

Linezolid resistance among multidrug resistant tuberculosis isolates: Insights from North Karnataka, India

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Abstract

Background: Drug-resistant tuberculosis (TB) is a serious threat to public health around the world. Treatment regimens have become more complex due to the growing number of reports of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) worldwide. An increasing issue is the emergence of linezolid resistance in clinical isolates, despite the oxazolidinone antibiotic's promising effectiveness against MDR-TB.
Aim & Objective: This study investigates the prevalence and mechanisms of linezolid resistance among MDR-TB isolates from North Karnataka, India.
Materials and Methods: In this study, all of the investigations involving culture-positive specimens were conducted in a Class-II biosafety cabinet in a biosafety level III laboratory. MGIT-based susceptibility testing was used to determine the LZD susceptibility for 308 isolates.
Results: A total of 11104 diagnostic samples were tested for First Line LPA at the C &DST Lab KMCRI, Hubli. From January 2023 to December 2023 and the results revealed 6.1% were found to be resistant to LZD among 308 MDR TB isolates.
Conclusion-This underscores the need for continuous surveillance, careful management of linezolid use, and the development of alternative therapeutic strategies. These findings contribute to the growing body of knowledge on TB drug resistance and inform future efforts to combat MDR-TB in the region.

Keywords: Linezolid Resistance, MDR TB, Northern Karnataka

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1. Introduction

In high-burden nations, drug-resistant TB significantly affects health outcomes and costs, and its prevalence is predicted to rise over the next 20 years.[1] The rise in extensively drug-resistant (XDR) TB is defined as TB that is resistant to fluoroquinolones, any Group A drug (LZD & BDQ), rifampicin, and isoniazid.(referred to as multidrug-resistant TB or MDR-TB), has highlighted the urgent need for new active medications to treat drug-resistant TB. In China, India, Africa, and Eastern Europe, cases of XDR and completely drug-resistant TB have been reported. This poses a growing risk to public health, urging the creation of innovative medications and dosage guidelines for effective treatment.[2]

Linezolid (LZD), the first oxazolidinone licensed for medicinal usage, was originally used to treat gram-positive bacterial infections. It was later shown to treble the percentage of treatment success for TB that is multidrug-resistant (MDR).[3]

Linezolid (LZD) was licensed by the Food and Drug Administration (FDA) in 2000 to treat Gram-positive infections. It is among the anti-TB drugs with the most potent effect on *Mycobacterium tuberculosis* (MTB).[7]LZD has thus been incorporated into national TB elimination programs on a larger scale and plays a significant role as an anti-TB agent. LZD reduces bacterial protein synthesis by binding to the 50S ribosomal subunit at its contact with the 30S unit, preventing the formation of a 70S initiation complex. As a result, resistance to LZD is anticipated to develop gradually and slowly.[2]

The WHO has suggested new treatment plans for MDR-TB programs that include bedaquiline (BDQ), pretomanid,

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and LZD with or without moxifloxacin (BPaLM/BPaL). Regrettably, resistance to LZD has been noted worldwide, especially in India, where MDR-TB is prevalent. A recent meta research indicated that the pooled prevalence of LZD resistance in clinical isolates of MDR-TB bacteria was 4.2%. Other risk factors for acquired LZD resistance include adding LZD to an inadequate or failed treatment, or stopping LZD due to side effects or loss to follow-up.[4]

The Nix-TB phase 3 clinical trial, which had 107 South African patients with drug-resistant TB, was the first to use the BPaL regimen. The ZeNix experiment came next, with 181 individuals enrolled from South Africa, Georgia, Russia, and Moldova.[8] A different protein synthesis inhibitor of *Mycobacterium tuberculosis* (MTB), spectinamide 1599, was added to the BPa (bedaquiline Pa spectinamide regimen) regimen because it had fewer side effects than the long-term use of the protein synthesis inhibitor LZD.[9] The safety of different doses of LZD combined with bedaquiline and Pa in people with pre- extensively drug-resistant (XDR) or treatment-intolerant MDR pulmonary tuberculosis is being investigated in India using a modified BPaL experiment.

Currently, the gold standard test for detecting LZD resistance is an automated phenotypic culture-based approach that uses the BACTEC MGIT 960 system at a critical concentration of 1µg/mL. Therefore, in situations when resistance to rifampicin and/or isoniazid is identified by nucleic acid amplification tests or LPA, the recommendations for programmatic management of drug resistant tuberculosis (PMDT) advise performing phenotypic LC-DST for determining LZD susceptibility. The main drawback of LC DST is that susceptibility report are not available for weeks or months, which causes a delay in the proper initiation of treatment.[10]

Early identification of LZD resistance would assist physicians to design a patient-specific regimen in accordance with the WHO's recommended drug replacement sequence. The inability of LC-DST to identify clinically significant resistance in some isolates that has been noted for the first-line anti-tubercular drugs is another drawback, in addition to its delayed results. In these situations, it suggests that genetic identification of drug resistance is a more accurate way to predict patient outcomes. Although this occurrence is unknown, it might also happen with LZD LC-DST. The WHO-approved commercial molecular techniques that are currently on availability, such as GeneXpert, TrueNat, and line probe tests, are not standardised for identifying LZD resistance.[3]

Therefore, the aim of the study is to know the rate of occurrence of LZD resistance among MDR-TB isolates from various clinical specimens.

2. Materials and Methods

This study was conducted at the C & DST Lab KMCRI, Hubli. A total of 307 drug-resistant *M. tuberculosis* isolates were collected between January and December of 2023. In this study, all of the investigations involving culture-positive specimens were conducted in a Class-II biosafety cabinet in a biosafety level III laboratory. The NALC-NaOH decontamination procedure was used to treat the sputum samples in accordance with WHO standards (final NaOH concentration, 1%). DNA was extracted from each individual sample using the GenoLyse® PCR CE/IVD Germany as directed by the manufacturer.

To accurately identify *M. tuberculosis*, on using the MTBDRplus V.2 assay for interpreting in compliance with the manufacturer's recommendations. The hybridisation strips clearly showed drug resistance by identifying specific mutant bands or by not having wild-type (WT) bands, which confirmed drug resistance.

The samples were then cultured using BACTEC™ MGIT™ 960 culture tubes. For rapid detection of *M. tuberculosis* complex, the Bioline™ TB Ag MPT64 test is used.

2.1. Phenotypic drug susceptibility testing

It was carried out using the BACTEC MGIT 960 system (Becton Dickinson, USA) in accordance with the WHO's recommended procedures and the diagnostic algorithm provided by the NTEP 2021 guidelines.1.0 µg/mL was used as the key value to determine LZD susceptibility. The isolates were used at an inoculum equivalent to one McFarland standard.

3. Results

11,104 diagnostic samples were tested for First Line LPA at the C &DST Lab KMCRI, Hubli. From January 2023 to December 2023. All samples were taken from a patient who had symptoms that pointed to active pulmonary tuberculosis. Of the 11,104 samples, 308 (2.7%) were found to be MDR. All 308 (MDR) samples were then cultured using the BACTEC™ MGIT™ 960 method. The *M. tuberculosis* complex is rapidly detected using the Bioline™ TB Ag MPT64 test.

MGIT-based susceptibility testing was used to determine the LZD resistance for 308 isolates. Of the 308 isolates, 19 (6.1%) were found to be resistant to LZD. Among the 19 resistant patients 12 were men & 7 were women.(**Figure 1**)

Among the 19 LZD resistant patients in our study, 12(63.1%) were cured, 3(15.7%) died, 3(15.7%) were lost to follow up & 1(5.2%) had their regimens changed.(**Table 1**)

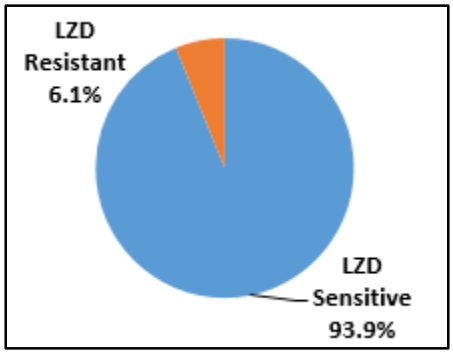

Figure 1: Graphical presention of LZD Resistance

Table 1: Representing the outcomes of the study

Outcomes	Percentage
Cured	12(63.1%)
Died	3(15.7%)
Lost to follow up	3(15.7%)
Regimens changed	1(5.2%)

4. Discussion

Drug-resistant TB is being treated with LZD, which is being seen as a significant advancement. Globally, however, reports of baseline resistance to LZD have been reported. Overall frequency among clinical isolates of MDR-TB is estimated to be 4.2%, with significant geographic variation in LZD resistance ranging from 22.2% in Spain to 0.2% in the US, according to a recent meta-analysis.[3]

A study conducted by Remya Nambiar, Jeffrey A. Tornheim*et al*., in Mumbai India(2021)[2] shows the resistance rate of 3.1% to LZD, in a another study conducted by Senjuti Sengupta, Parul Jain *et al*., in Uttar Pradesh, India(2023)[3] shows the resistance rate of 5.31% LZD, in contrast present study shows the resistance rate of 6.1% to LZD.

The results of a longer oral regimen including BDQ and a shorter oral regimen have been compared in studies. The benefits of a shorter, all-oral BDQ regimen were greater than those of a longer, injectable free BDQ regimen.

Of the 19 patients with LZD resistance, 3 (15.7%) died as a result of co-morbid conditions such diabetes, HIV, and ageing. One patient's medication was altered since he was exhibiting adverse side effects to some of the medication.

WHO has published the 2021–2025 Global Lists of High Burden Countries for MDR/RR TB. The top 10 countries with a severe burden of incident rate and the top 20 countries with an estimated absolute number of incident cases are included in the list, which has a total of 30 countries. While some nations, like Ethiopia, were removed from the list, others, like Nepal, have been added to it in comparison to 2016–2020. From 2016 to 2018, 29% of patients with conventional XDR-TB who were treated (without BDQ) had an average success rate. In 2018, this rose to 48 percent. One major obstacle to TB elimination is managing MDR-TB and XDR-TB.[10]

For a number of reasons, individuals receiving shorter regimens of DR-TB treatment with more recent oral medications are anticipated to have a higher quality of life than those receiving longer or standard DR-TB regimens. Injectable-free treatment increases patient adherence and makes community program implementation easier.[5]

The WHO TB mutation catalogue 2021 provides recommendations for single-nucleotide changes that are highly indicative of anti-tuberculosis medication resistance.[11] Drug resistance increases the likelihood of less favourable treatment outcomes and lengthens the course of treatment for tuberculosis.[12] When a clinical isolate of MTB has more resistance indicators than the threshold for XDR TB, patients are at a much higher risk of treatment failure and death. Although LZD-based combination therapy has a significant risk of damage, including peripheral neuropathy and myelosuppression, there are findings that indicate this medication may enhance survival outcomes and culture conversion rates[13]. Due to the extended period of MDR-TB therapy and the comprehensive daily medication regimen, there is an increased risk of adverse drug responses, including ototoxicity and mild to severe gastrointestinal problems. Given the MTBC's slow growth and the high expenses and intricate infrastructure needed for traditional pDST, rapid genotypic DST provides a more useful and effective way to get drug-susceptibility results that is crucial for patient care.[3]

It is obvious that combination therapy is essential for stopping the emergence of organisms that are resistant to drugs. Remarkably, MDR-TB treated with BDQ-LZD showed a low rate of acquired LZD resistance. First, there is no overlap in the molecular processes underlying MTB resistance to BDQ and LZD during treatment. Thus, in theory, BDQ in combination therapy should continue to be effective against LZD-resistant mutants, thereby preventing the accumulation of mutations that give LZD resistance. According to a retrospective clinical study, the earliest reported occurrence of LZD resistance in MDR-TB patients was 7 months after beginning LZD therapy, while the median period until LDZ resistance manifested was 22 months.[15]

85% of MDR-TB patients receiving BDQ-containing regimens showed culture conversion at 20 weeks, which was caused by the potent bactericidal activity of BDQ. Decreased time to sputum culture conversion thus further inhibited the development of LZD-resistant organisms. Despite the lack of clarity regarding the precise mechanism of action, the results support recent WHO treatment recommendations that prioritise the BDQ-linezolid combination as an MDR-TB regimen. Meanwhile, this combination should be supplemented with two or more other medications, such as fluoroquinolones and clofazimine, to prevent drug resistance and clinical failures.

LZD resistance14 may also result from cellular changes that negatively impact cell permeability to antimicrobial medications, such as resistance mechanisms involving efflux pumps and changes in cell wall thickness. The addition of an efflux pump inhibitor significantly decreased MTB sensitivity to LZD. Researchers thus postulate that an active ejection mechanism may serve as a non-ribosomal resistance mechanism in MTB strains with somewhat high MICs. Additionally, an electron microscopic examination of the ultrastructure of the mycobacterial cell wall revealed a favourable correlation between the thickness of the cell wall in MTB isolates and widespread treatment resistance.

Consequently, decreased permeability of the mycobacterial cell wall may be another mechanism contributing to LZD resistance, given that all research participants had MDR- and XDR-TB.

Therefore, more in-depth investigation is required to understand the molecular mechanisms granting MTB isolates resistance to LZD.[6]

5. Conclusion

It is alarming that 6.1% of the TB patients have resistance to LZD in our study. Because of the high level of LZD resistance, it is essential to do DST for all the patients who have put on LZD therapy. Moreover, there is little correlation between pDST and genotypic nanopore sequencing resistance identification for LZD. To be incorporated into the bioinformatics pipeline, LZD mutations in Indian isolates require additional research and cataloguing. To potentially explain phenotypic LZD resistance, further mechanisms of LZD resistance should be investigated.

6. Ethics Statement

The authors of this manuscript declare that this scientific work complies with reporting quality, formatting and reproducibility guidelines set forth by the EQUATOR Network. The authors also attest that this study was determined to require the Institutional Ethics Committee review, and the corresponding approval number is JSS/MC/PG/0040/2022-23 Dated 05.04.2023. The authors have not registered this study with the Clinical Trial Registry as it is not applicable.

7. Author Contributions

NRD collected the samples, NRD performed the preliminary and main tests, NRD, TA, RBN & N analysed the data, NRD, TA, RBN & N constructed the agreements and errors.

8. Abbreviations

BDQ- Bedaquiline; **BDQ-R TB**- Bedaquiline-resistant tuberculosis; **BPaLM** -Bedaquiline (BDQ), pretomanid, LZD and moxifloxacin; **C &DST**- Culture & Drug Susceptibility Testing; **DST** -Drug susceptibility testing; **DR**-Drug resistant; **DR-TB**- Drug resistant tuberculosis; **FDA** -Food and Drug Administration; **LC-DST**-Liquid Culture Drug susceptibility testing; **LZD**- Linezolid; **MDR-TB**-multi-drug resistant tuberculosis; **MGIT**- Mycobacteria Growth Indicator Tube; **MTB**-Mycobacterium tuberculosis; **NALC**- N Acetyl L Cysteine; **NaOH** -Sodium hydroxide; **PMDT**-Programmatic management of drug resistant tuberculosis; **RR-TB**- Rifampicin Resistance tuberculosis; **TB**- Tuberculosis; **WHO**- World Health Organisation; **WT** -Wild-type; **XDR** -Extensively drug-resistant.

9. Conflict of Interest

None.

10. Conflict of Interest

None.

Reference

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