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Original Research Article

Bedaquiline resistant tuberculosis: Implication in North Karnataka, India

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Abstract

Background: The treatment of multi-drug resistant tuberculosis (MDR-TB) is becoming increasingly difficult due to bedaquiline-resistant tuberculosis (BDQ-R TB), especially in areas like Karnataka, India. MDR-TB is treated with BDQ, an innovative drug, but the effectiveness of current treatment plans is in jeopardy due to its growing resistance. The increasing incidence of drug-resistant tuberculosis in Karnataka has sparked worries due to the state's high BDQ-R TB prevalence. Resistant strains are becoming more prevalent due to a number of factors, including inadequate diagnostic infrastructure, poor drug adherence, and incomplete treatment courses.

Aim and Objective: To know the rate of occurrence of bedaquiline resistance among MDR/RR TB isolates.

Materials and Methods: 307 drug-resistant TB isolates of *M. tuberculosis* were collected from C & DST Lab KMCRI, Hubli, between October 2023 to October 2024. Bed aquiline resistance among MDR/RR TB isolates were determined by DST method.

Results: 256 isolates were tested for BDQ susceptibility using MGIT-based susceptibility testing. 14 (5.4%) of the 256 isolates were found to be BDQ resistant. Ten of the 14 patients with BDQ resistance were men, and four were women.

Conclusion: This abstract provides insight on how BDQ resistance affects MDR-TB treatment and control in Karnataka, highlighting the pressing need for enhanced diagnoses, better surveillance systems, and efficient treatment plans. The results highlight the significance of BDQ resistance management in preventing the spread of highly resistant TB and improving the prognosis of MDR-TB patients in the area.

Keywords: Bedaquiline, Multi-drug resistant tuberculosis, Rifampicin-resistant tuberculosis, Drug susceptibility testing.

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1. Introduction

Tuberculosis is a disease that may be prevented and is typically cured. Nevertheless, after three years in which corona virus disease (COVID-19) supplanted TB as the world's greatest infectious agent, TB likely regained its position in 2023, accounting for nearly twice as many fatalities as HIV/AIDS. Over 10 million people still have TB each year, and that figure has been increasing since 2021. The World Health Organisation (WHO) and the United Nations (UN) have agreed that all Member States must take immediate action to eliminate the worldwide tuberculosis pandemic by 2030.

The organism that causes tuberculosis, *mycobacterium tuberculosis*, is released into the air by TB patients when they cough or otherwise exhale. Nearly 25% of the populations worldwide are believed to have developed tuberculosis. Within the first two years after infection, the risk of contracting TB peaks at around 5%, after which it drastically declines. Men are more likely than women to get tuberculosis (TB) illness, with around 90% of cases occurring in adults. Although the illness usually affects the lungs (pulmonary tuberculosis), it can also affect other parts of the body.

Nearly 50% of people die from tuberculosis disease if they do not receive treatment. Approximately 85% of TB

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patients may be treated with the current WHO-recommended anti-TB medication regimen, which lasts four to six months. Treatments for TB infections range from one to six months. All individuals who require treatment for tuberculosis (TB) infection or sickness must have access to universal health coverage (UHC). Additionally, multispectral intervention to address TB factors such poverty, under nutrition, HIV infection, smoking, and diabetes can help lower the number of individuals who become infected and develop illness (and consequently, the number of fatalities caused by TB).

A few nations have already managed to lower their annual TB disease burden to less than 10 cases and one fatality per 100,000 people. To quickly bring the number of cases and fatalities worldwide down to the levels already attained in these low-burden nations, scientific advances (such as a new vaccine) are required.

In recent years, political commitment to eliminating the TB pandemic has increased. Two high-level UN meetings on tuberculosis have been held: one in 2018 and the second in 2023. The political statement at the 2023 summit contained additional aims and pledges for the years 2023–2027 in addition to reaffirming those already established in the WHO end TB strategy and the UN Sustainable Development Goals (SDGs).

In 2023, 3.2% of new cases and 16% of patients who had previously undergone treatment were predicted to have MDR/RR-TB; in 2015, the corresponding numbers were 4.1% and 20%.

Five nations were predicted to account for more than half of all MDR/RR-TB infections globally in 2023: China (7.3%), Indonesia (7.4%), the Russian Federation (7.4%), India (27%), and the Philippines (7.2%). The largest proportions of TB patients with MDR/RR-TB (>50% of previously treated cases) were seen in the Russian Federation, many Eastern European nations, and Central Asian nations.[1]

A major obstacle to the objective of eliminating tuberculosis by 2035 is drug-resistant TB. The new drug bedaquiline (BDQ) was touted as a promising treatment for drug-resistant (DR) TB. In 2012, it was approved by the US Food and Drug Administration (FDA) to treat multidrug-resistant (MDR) TB. Under the Central TB Division Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India's Revised National TB Control Program, the medicine is presently available in India as part of the BDQ condition access program for the treatment of drug-resistant TB.[12,13]

In India, BDQ is provided free of charge with conditional access through the National TB program. It is administered in addition to the standard DR-TB treatment. The recommended dosage is 400 mg once daily for two weeks, followed by 200 mg three times a week for 22 weeks. The MDR-TB regimen should be continued after 24 weeks of BDQ therapy, as per the national TB treatment guidelines.[11]

BDQ is an anti-mycobacterial drug that belongs to the di-arylquinolines group and exhibits strong bactericidal activity against both replication and nonreplicating mycobacteria. Bacterial ATP production is decreased when BDQ inhibits the action of ATP-synthase.[2]

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

2. Materials and Methods

2.1. Sample collection and processing

In this study 307 drug-resistant TB isolates of *M. tuberculosis* were collected from C & DST Lab KMCRI, Hubli, between October 2023 to October 2024. The study was approved by Institutional Ethics Committee. (Ref No: JSS/MC/PG/0040/2022-23 Dated 05.04.2023). In this study, clinical isolates of individuals who were not yet exposed to BDQ in their regimen were collected. In a Biosafety level III laboratory, all of the investigations involving culture-positive specimens were handled in biosafety cabinet Class-II. The sputum samples were processed using the NALC-NaOH decontamination method in compliance with WHO guidelines (final NaOH concentration, 1%).[3] DNA was extracted from each individual sample using the GenoLyse® PCR CE/IVD Germany as directed by the manufacturer.[4]

Following the manufacturer's recommendations for interpretation, the MTBDRplus V.2 test was used to accurately identify *M. tuberculosis*. Through the identification of certain mutant bands or the lack of wild-type (WT) bands, which validated drug susceptibility, the hybridisation strips strongly demonstrated drug resistance.

The samples were then cultured using BACTEC™ MGIT™ 960 culture tubes. For rapid detection of *M. tuberculosis* complex, the Bioline™ Ag MPT64 test was used.[4]

2.2. Direct susceptibility testing using Mgit 960

The remaining pellet from the MTB growth positive tube was used as the inoculum for BDQ susceptibility testing, following the manufacturer's instructions for the BDQ M960 assay, and was suspended in phosphate buffer (pH 6.8) until it reached a final volume of 2 ml. The control tube, which also contained polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin [PANTA], was inoculated with 0.5 ml of the resuspended pellet diluted 1/100. The tube containing 100 g/ml BDQ, which also contained PANTA, was inoculated with 0.5 ml of the undiluted resuspended pellet. Tubes were cultivated using the 13-day BDQ susceptibility testing protocol in the Bactec 960 MGIT device. The direct DST findings from the MGIT device were classified as either susceptible (S) or resistant (R).

Each lot of MGIT 960 BDQ medium and drugs set was tested for the *M. tuberculosis* H37Rv strain as a quality control measure. By cultivating on blood agar, all MGIT tubes that were positive were examined for contamination. Ziehl-Neelson stain was used to verify that acid-fast bacilli were present in the smear, whereas Gram stain was employed to rule out contamination.

3. Results

10928 diagnostic samples were tested for first line LPA at the C & DST Lab KMCRI, Hubli. From October 2023 until October 2024. The clinical and demographic information was gathered from patients who had symptoms that pointed to active pulmonary tuberculosis in all of the samples. 307 (2.8%) out of 10928 samples had been found to be RR/MDR. The BACTEC™ MGIT™ 960 technique was then used to cultivate all 307 (RR/MDR) samples. For quick identification of the *M. tuberculosis* complex, the Bioline™ TB Ag MPT64 test is performed.

51 samples out of 307 had been identified to be contamnated. Using MGIT-based susceptibility testing, the BDQ susceptibility of the remaining 256 isolates was examined. Of the 256 isolates, 14 (5.4%) were found to be resistant to BDQ. Of the 14 BDQ-resistant patients, four were female and 10 were male.

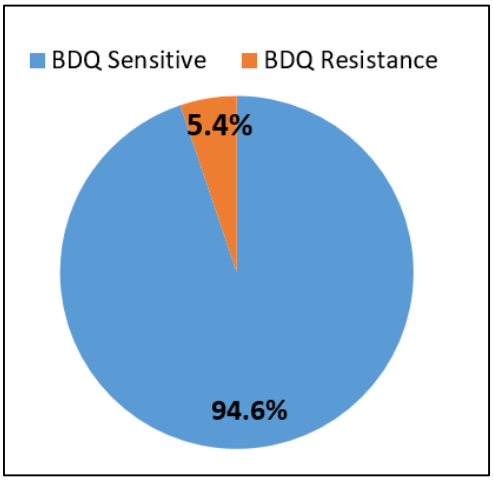

Figure 1: Representing the BDQ resistance among RR/MDR isolates

4. Discussion

The introduction of new and repurposed anti-TB drugs gives hope to DR-TB patients who have few treatment options, but the benefits are being offset by the rapid development of resistance.[5] Despite BDQ's demonstrated great efficacy in treating MDR-TB, its improper or insufficient use may cause resistance strains to arise. We conducted drug susceptibility testing to analyse the BDQ resistance in Karnataka, India, as there have been few studies that have examined the resistance status of MDR-TB against BDQ.[6]

In recent years, there have been reports of BDQ-based therapy regimens failing clinically worldwide. Most of these patients were found to be resistant due to a mutation in the Rv0678 gene, which is connected to efflux pump pathways.[2] In China, Yan Hu et al study revealed that the resistance rate of MDR-TB to BDQ was 4.4%.[6] In Russia, Tatiana Umpeleva *et al* study from 2023 revealed that the resistance rate of MDR-TB to BDQ was 2.1%.[7] In contrast, the current study reveals that the resistance rate of MDR-TB to BDQ is 5.4%.

Seven of the 14 BDQ-resistant patients in this study are receiving modified therapy with clinical improvement, four patients have died, and three patients have been cured.

According to Yang *et al.*, BDQ resistance may develop spontaneously, during therapy with other anti-TB medications, or as a result of prior usage of antifungal medications. According to Wu *et al.*, the 4% prevalence rate of azole-resistant Aspergillus fumigatus clinical isolates in Taiwan primarily arose from the environment and during antifungal treatment, and its relationship to BDQ resistance is still unclear. The future use of BDQ in the treatment of DR-TB is challenged by the unidentified cause of pre-existing BDQ resistance.[8]

However, the two main mechanisms of BDQ resistance, which are already widely distributed, are target modification (mutations in atpE, which codes for the target) and over-expression of an efflux pump (duplications in the Rv0678 gene, which codes for the repressor of MmpS5-MmpL5 efflux system). Even when BDQ-resistance is low, several reports have shown that mutations in the Rv0678 gene represent the most common route of BDQ resistance in clinical settings.[9]

In order to battle BDQ-resistance caused by Rv0678 mutations and make BDQ available to the maximum number of patients, verapamil, an efflux inhibitor, has been demonstrated to increase the efficiency of BDQ against both Mycobacterium abscessus and *M. tuberculosis*.[14]

The fact that the baseline isolates were sensitive to bedaquiline suggests that the effectiveness of treatments using bedaquiline depends on a functional background regimen that eliminates underlying antibiotic resistance. Pulmonary cavities are another significant feature linked to treatment outcomes. These cavities further encourage the development of drugs resistance, most likely as a result of increased bacterial loads.[15] If the cavities are bigger, drugs might not be able to get to the central lesion with the highest bacterial load. For example, bedaquiline accumulates in cellular regions of a granuloma, whereas clofazimine is unable to reach the necrotic centre of caseous lesions. In contrast, linezolid and moxifloxacin effectively penetrate all lesion types. Cavities therefore provide a microenvironment with varying drug doses for the infecting MTBC strain. Specifically, bedaquiline levels may briefly fall below levels

that are effective. This changing microenvironment may select for distinct subpopulations of bedaquiline-resistant clones, although susceptible bacteria with unaltered canonical resistance genes still remain.[10]

Quick genotypic tests based on sputum to rule out for WHO group A drug bedaquiline and linezolid are lacking makes it more difficult to diagnose MDR-TB and rifampicin-resistant TB. New methods like early targeted next-generation sequencing, like the Deeplex test, or even WGS of the MTBC genome directly from patient specimens may improve the treatment of MDR-TB patients in high-burden countries.[10]

5. Conclusion

This study has determined BDQ resistance in 5.4% of the MDR-TB strains in North Western part of Karnataka. Emergence of BDQ-resistant *M.tuberculosis* strains pose threat to the success of current treatment regimens. Timely and precise diagnostic methods are crucial to help RR/MDR-TB patients have better treatment results and stop the spread of drug-resistant strains. In order to detect the resistance profiles of the pathogens and ensure that patients receive the most effective treatment combinations, drug susceptibility testing (DST) that is both genotypic and phenotypic is essential. Regular and comprehensive testing allows for more personalized treatment plans and helps in preventing further resistance development. The availability of alternative, effective treatment regimens tailored to individual resistance patterns is critical to controlling MDRTB in India. Strengthening diagnostic infrastructure and enhancing access to DST can significantly improve treatment success and reduce the prevalence of resistant TB strains, contributing to more sustainable control of the disease.

6. Research Quality and 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. Source of Funding

Self.

8. Conflict of Interest

None

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