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Original Research Paper

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DETERMINATION OF SENSITIVITY TO LINEZOLID IN DRUG-RESISTANT TUBERCULOSIS CASES

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ABSTRACT

Introduction: Tuberculosis is caused by Mycobacterium tuberculosis and remains a leading infectious cause of mortality globally. Linezolid, an oxazolidinone, has strong in vitro evidence of action against drug resistant isolates of M. tuberculosis. It can be administered orally or intravenously and has been shown to be highly beneficial if used for at least six months. **Aim:** To determine the sensitivity of linezolid in patients with Drug-resistant tuberculosis. **Methods:** An 18-month observational study was conducted in the TB Culture and Drug Sensitivity Screening lab of the Department of Microbiology at the Tertiary Care Center in Mumbai on a sample size of 1031 patients. Samples from pulmonary and extrapulmonary tuberculosis patients of MDR/XDR (confirmed by LPA) cases were collected for phenotypic DST for Linezolid utilizing the automated BACTEC MGIT 960 system in a biosafety level 3 lab. **Results:** Out of 1031 patients, 88.9% were sensitive to Linezolid, and 11.1% were resistant. Linezolid sensitivity for MDR-TB patients was 88.72% of the cases being sensitive, and 11.28% resistant. Linezolid sensitivity in pre-extensively drug-resistant tuberculosis was 88.27% and 11.73% were resistant. Among 1031 samples, 50 samples taken for Linezolid and Bedaquiline liquid DST, of which 15 were XDR and 4 samples among these 15 XDR samples were sensitive to Linezolid **Conclusion:** The study concluded that Linezolid is an effective drug used in the NTEP for the treatment of M/XDR-TB, H mono/poly DRTB, as well as the newer BPaL regimen. The results showed an overall sensitivity of 88.9%.

KEYWORDS : linezolid sensitivity, drug resistant tuberculosis, BACTEC MGIT960, Bedaquiline

INTRODUCTION

Tuberculosis (TB) is caused by infection with bacteria of the Mycobacterium tuberculosis complex. Pulmonary tuberculosis (PTB) is the most common form of tuberculosis which is transmitted by aerosolized droplets from people with active pulmonary tuberculosis when they cough. WHO End TB strategy calls for early diagnosis and universal access to Drug Sensitivity Testing (DST) and is the mainstay of TB control worldwide and in India (NTEP) also. The control of TB is hampered by the emergence of multidrug-resistant TB (MDR-TB), pre-XDR [MTB which is MDRTB/RR-TB and resistant to any quinolone (FQ)] and extensively drug resistant TB (XDR-TB) [MDRTB and resistant any quinolone (levofloxacin or moxifloxacin) and atleast one additional Group A drug (presently Bedaquiline (Bdq) or Linezolid or both)]. [1,2].

Linezolid is an oxazolidinone antibiotic that disrupts protein synthesis by binding to the 70S initiation complex of bacterial ribosomes[3]. It also binds to human mitochondria and inhibits protein synthesis, which is the mechanism of toxicity in clinical use[4]. It is active against most gram positive bacteria, with extensive evidence of in vitro activity against isolates of M. tuberculosis, including those resistant to first-line drugs[5,6].

Linezolid improved outcomes of drug resistant TB in clinical trials.WHO recommended Linezolid as a preferred agent for all patients with drug-resistant TB in 2018. Linezolid was categorized as a 'Group 5' drug in 2011 WHO Drug-resistant TB guidelines[7]. However, the 2016 WHO update re-allocated as 'Group C: other core second line agent', prioritizing its use over some more traditional agents [8]. In 2018, in a rapid communication from the WHO on treatment of MDR-TB and Rifampicin-monoresistant-TB, Linezolid's position was further upgraded to 'Group A: medicines to be prioritised [9], suggesting that it should be included in the regimen for all patients unless contraindicated.

Linezolid can be taken orally or intravenously. Its excellent oral bioavailability is an advantage, avoiding the need for long-term daily injections [10]. Though an adult dose of 600mg twice daily is commonly used for upto 28 days to treat infections due to gram positive bacteria, a variety of dosing strategies have been used in the context of drug-resistant tuberculosis, where treatment duration is much longer. These have ranged from 300mg to 1200mg daily, with once or twice daily administration. Lower doses have been tried in an attempt to increase tolerability and reduce toxicity[11,12]. The optimal dosing and duration of linezolid remains unclear from the perspective of preventing emergence of resistance, as well as efficacy, tolerability and toxicity. Adverse effects of Linezolid include suppression of bone marrow causing anemia and thrombocytopenia, peripheral neuropathy, And optic neuropathy leading to disability and blindness. [13].

The End TB strategy calls for early diagnosis and prompt treatment of all persons of all ages with any form of drug-susceptible or drug resistant TB. The effective management of multi-drug and extensively-drug resistant TB (M/XDR-TB) relies upon the rapid diagnosis and treatment of resistant infections. Culture-based phenotypic drug susceptibility testing (DST) methods are currently the gold standard for drug resistance detection [2].Drug sensitivity testing uses critical concentrations of anti-TB agents to determine the susceptibility or resistance of Mycobacterium tuberculosis complex. The critical concentration is defined as the lowest concentration of an anti-TB agent in vitro that will inhibit the growth of 99% of phenotypically wild type strains of M.tuberculosis complex.

Laboratory tests of the sensitivity of tubercle bacilli to anti-tuberculosis agents serve three main purposes. Firstly, they can be used as guidance in the choice of chemotherapy to be given to a patient. Secondly, they are of value in confirming that drug resistance has emerged when a patient failed to show a satisfactory response to treatment and thirdly can be used for the surveillance of emerging drug resistance.

METHODS AND MATERIALS

This was a prospective observational study of all samples of Drug resistant TB cases of pulmonary and extra-pulmonary tuberculosis for a period of 18 months starting from January 2021 to June 2022 conducted in the TB Culture and Drug

Sensitivity Testing lab of Department of Microbiology, Tertiary Care Centre, Mumbai.

Inclusion Criteria:

- All samples from MDR/PRE-XDR/XDR cases of pulmonary (PTB) and extra-pulmonary tuberculosis (EPTB) referred to the C-DST for LC DST by the National TB Elimination Program cultured by MGIT and showing growth for M tuberculosis.

Exclusion Criteria:

- Cases of Drug sensitive Mycobacterium tuberculosis (DSTB)
- Cases of Non tuberculous mycobacteria (NTM)

All DRTB culture isolates (confirmed by LPA) were taken for Liquid Culture DST for Linezolid using the automated BACTEC MGIT 960 system based on the principle that an increase in the fluorescence in the sensor due to the utilization of oxygen by MTB is measured automatically and designated as Growth Unit (GU). Samples are incubated in MGIT until the instrument reports the DST test complete (3-14 days). The critical concentration for Linezolid is 1µg/ml. When GU of the Growth control reaches 400, GU values of the drug containing tubes found $\leq$ 100 are considered **susceptible** while >100 are considered **resistant** to linezolid[14].

Ethical approval

No consent was required as samples processed were directly received in the laboratory as per NTEP guidelines and were blind-coded for this research study.

RESULTS

Out of 1031 patients, 56.74% were female and 43.06% were male patients while 0.2% were transgender. Majority of the patients (68.57%) were in the age group of 19-59 years, followed by 19.89% in the age group of 13-18 years. 88.9% of them tested responsive to Linezolid and 11.1% resistant.

Table 1: Sensitivity to Linezolid in DRTB Patients (n=1031)

Linezolid Sensitivity	Number	Percentage
Sensitive	916	88.90%
Resistant	115	11.10%

Total 993 MDR-TB cases were divided into 88.72% sensitive and 11.28% resistance to linezolid cases. Linezolid sensitivity was 88.14 % in males, 89.13 % in females, 100% in transgender individuals.

Males, females, & transgender people did not differ significantly from one another (p=0.462). Linezolid sensitivity in cases of pre-extensively drug-resistant tuberculosis (n=946) was 88.27% whereas 11.73% were Linezolid resistant. Males, females, & transgender individuals' intergroup comparisons were not statistically significant (p=0.863).

Table 2: Linezolid sensitivity for category-wise Drug Resistant TB Patients

Category	Total	Linezolid Sensitive	Linezolid Resistant
MDR (Rif &Inh resistant + any/all FQ or any other anti-TB drug resistant)	993 (Only Rif &Inh resistant= 59,Rif & inh+FQ Resistant=934)	881 (88.72%) (Only Rif &Inh resistant= 56,Rif & inh+FQ Resistant=825)	112 (11.28%) (Only Rif &Inh resistant= 03, Rif & inh+FQ Resistant=109)
Pre-XDR (MDR/RR+ any FQ resistant)	946 MDR+FQ Resistant=934, RR+FQ Resistant=12)	835 (88.27%) (MDR+FQ Resistant=825, RR+FQ Resistant=10)	111(11.73%) (MDR+FQ Resistant=109, RR+FQ Resistant=02)
Rifampicin Sensitive + Isoniazid Resistant+ FQ Resistant	24	23 (95.83%)	01(4.17%)
Mono-Rif resistant	01	01 (100%)	-
Mono-Inh resistant	01	01 (100%)	-

Among 1031 samples, 111 were MDR/RR, fluoroquinolone and Linezolid resistant i.e. XDR TB cases. 50 samples out of 1031 were taken for both Linezolid and BDQ liquid DST, of which 15 were XDR-TB cases and 4 samples among these 15 XDR samples were sensitive to Linezolid.

Table no: 3 Linezolid sensitivity in XDR-TB Cases (n=1031)

TOTAL SAMPLES=1031	
MDR/RR+FQ Resistant+Linezolid Resistant	111 (10.08%)
Total=15/50	
Only BDQ Resistant	04 (26.66%)
Only Linezolid Resistant	06 (40%)
BDQ + Linezolid Resistant	05 (33.34%)

DISCUSSION

In our study, using Linezolid as a single drug, has been exclusively to ascertain the sensitivity of MDR-TB and XDR-TB. However, its recent incorporation into Group A also highlights the rising importance of this drug. It is important that quality assured LCDST results are available for this drug.

In our study, 11.1% were linezolid resistant and 88.9% were linezolid sensitive.(Table no: 1). In comparison, Tornheim J et al, showed a Linezolid resistance was seen in 6.7% cases[15].

Linezolid resistance rates reported in studies of other areas with high prevalence of drug resistance such as Beijing (5.6%) and Karachi (5.9%), though other studies have reported linezolid resistance rates as high as 10.8% among MDR-TB isolates[16,17,18].

A comprehensive meta-analysis study by Azimi et al[19] revealed a LNZ resistance among clinical isolates of MDR-TB of 4.2%. LNZ resistance rate among the clinical isolates of MDR-TB in 14 different countries showed that Spain (22.2%) and United States (0.2%) had the highest and lowest prevalence rate, respectively.

Our study showed an 88.27% sensitivity of Linezolid in pre-XDR-TB cases.Linezolid sensitivity in pre-XDR-TB cases by Singla et al[20], Kwon et al.[21] in Korea , Mitnick et al. [22] in Peru , showed 70% , 67% in 27 patients and 60% in 48 patients respectively.

In our study, total number of cases with rifampicin, isoniazid, fluoroquinolone and Linezolid Resistance is 104. 50 samples out of 1031 were taken for Linezolid and BDQ liquid DST, of which 15 were XDR-TB cases and 4 samples among these 15 XDR samples were sensitive to Linezolid. Banka, et al found that of the 2750 samples that underwent 14-drug MGIT DST, 250 were XDR-TB, of which 44 (17.6%) showed resistance to LZD[23].

Out of 1031 culture isolates, 01 was Mono-Rifampicin resistant and 01 was Mono-Isoniazid resistant, both of which were sensitive to Linezolid.

Practical implementation of our study would include incorporation of Linezolid into established regimens and management of selected populations with these regimens. Then we would be able to curate linezolid regimens for the Indian population. Also, our data has been based on the findings of a single tertiary care hospital in Mumbai; it needs to be expanded to include pan-India results.

CONCLUSION

The study concluded that Linezolid is an effective drug used in

the NTEP for the treatment of M/XDR-TB, H mono/poly DRTB, as well as the newer BPaL regimen. The results showed an overall sensitivity of 88.9%. A total of 1031 samples underwent liquid culture and drug sensitivity testing for linezolid using a critical concentration of 1 mcg, as prescribed by the National TB Elimination Program of the Indian government. The quality of drug sensitivity testing was ensured by following the quality assurance program of NTEP and passing the proficiency testing protocol annually as per the Central TB Division of the Indian government. Linezolid is highly sensitive **But** significant percentage of resistance (11.1%) was seen. It is important to conduct further studies in this field to better understand the factors leading to linezolid resistance and to gather country-wide evidence of its resistance. It is also crucial to use this drug under proper cover of DST (drug sensitivity testing) to ensure its efficacy and prevent the development of resistance. Future perspectives suggest that developing new drugs or treatment regimens that are effective against drug-resistant TB strains and reducing the burden of drug-resistant TB will be a priority in the coming years.

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