

Evaluation of Sensitivity and Resistance of Linezolid in Pre Extensively Drug Resistance Tuberculosis and Extensively Drug Resistance Tuberculosis at a Tertiary Care Hospital, Jamnagar, Gujarat, India

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ABSTRACT

Introduction: The diseases have a high prevalence in India, accounting for one fourth of the Tuberculosis (TB) cases in the world. The situation becomes more complicated due to the rising of Human Immunodeficiency virus/AIDS pandemic, the emergence of Multidrug- Resistance (MDR) TB, Pre Extensively Drug Resistance (Pre-XDR) TB, and Extensively Drug Resistance (XDR) TB. Therefore, new anti-TB drugs are urgently needed to treat MDR-TB, Pre XDR-TB, and XDR-TB. Linezolid was the first oxazolidinone compound licensed for clinical use.

Aim: To evaluate the sensitivity and resistance of linezolid in Pre XDR-TB and XDR-TB patients at a tertiary care hospital, Jamnagar, Gujarat, India.

Materials and Methods: This was a prospective study conducted from May 2017 to January 2018. Total sample received were 590 MDR-TB suspected cases. All samples were proceeded for rapid detection of MDR of *Mycobacterium*

tuberculosis by direct Drug Susceptibility Testing (DST) using Bactec MGIT 960 system.

Results: Out of the total 590 samples, 468 were culture positive among this: 14 (3%) were linezolid resistance, 9 were Pre XDR-TB with linezolid resistance, 03 were XDR-TB with linezolid resistance and 2 were only linezolid resistance. A total of 454 (97%) were linezolid sensitive, 143 were Pre XDR-TB with linezolid sensitive and 19 were XDR-TB with linezolid sensitive. A total of 14 (3%) were HIV infected of which 2 had Pre XDR-TB and 1 had XDR-TB. All 14 were linezolid sensitive.

Conclusion: The present study showed that linezolid was found to be highly sensitive (97%) with low (3%) resistance in MDR-suspected patients. Linezolid is effective in treatment of MDR-TB, Pre XDR-TB and XDR-TB patient, but while receiving linezolid, patients should be closely monitored for signs or symptoms of bone marrow toxicity and peripheral and optic neuropathy.

Keywords: Human immunodeficiency virus, Multidrug resistance tuberculosis, *Mycobacterium growth indicator tube-960*, *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis is caused by *Mycobacterium tuberculosis*. Robert Koch isolated tuberculin bacilli in 1882. Tuberculosis is the second-most common cause of death from infectious diseases after those due to HIV/AIDS [1]. India is one of the high burden TB countries in the world [2]. According to the WHO Tuberculosis report, 2014, there were 9 million cases of TB globally. India having a share of 24% of the global burden [2].

The situation becomes more complicated due to the rising Human Immunodeficiency Virus/AIDS pandemic, the emergence of MDR-TB, Pre-XDR-TB, and XDR-TB. Therefore, new anti-TB drugs are urgently needed to treat MDR-TB, Pre XDR-TB, and XDR-TB. In 2016, Linezolid was reclassified and recommended by the World Health Organisation (WHO) as a core second-line agent in the MDR-TB regimen based on emerging evidence. It has shown statistically significant treatment benefits with its promising bactericidal activity and synergistic effect with other agents against resistant strains of *Mycobacterium tuberculosis* [3]. Linezolid was the first oxazolidinone compound licensed for clinical use. However, there are only few in-vitro studies of linezolid's activity against clinical *M. tuberculosis* isolates.

Antibiotic resistance is a growing problem in multiple drug resistance TB infections. Primary resistance occurs when person becomes infected with a resistance strain of TB, but a person with fully

susceptible TB may develop secondary (acquired) resistance during therapy because of inadequate treatment, not taking the prescribed regimes appropriately (lack of compliance), or using low-quality medications [4]. In XDR-TB patients, resistance of second line drug depends upon factors like previous TB treatment, Directly Observed Therapy System (DOTS), previous fluoroquinolone and second line drug like antibiotic exposure, MDR-TB treatment duration and surgical resection [5-7]. Key drugs for management of MDR-TB and XDR-TB patients are fluoroquinolone and second line injectable drug due to increases resistance of the drugs [8-11]. Several studies have shown increase in resistance of fluoroquinolone and injectable drug. So, other effective third line drugs like (Linezolid, Meropenem and Cotrimoxazole etc..) [12-15], are studied but present study was done on linezolid drug.

The aim of this study was to evaluate sensitivity and resistance of linezolid who have MDR-TB, Pre-XDR-TB and XDR-TB treatment. In India burden of XDR-TB patient and their treatment outcome are not yet known due to lack of quality assured laboratories for performing DST for 1st and 2nd line drugs against TB [16]. In recent year molecular methods for detection of drug resistance and liquid culture have also been described, such as the line probe assay for detection of 1st line and 2nd line drug resistance. The present study evaluated rapid, effective *Mycobacterium Growth Indicator Tube* (MGIT) method for DST of Linezolid on clinical isolates of *M. tuberculosis*.

Advantages of molecular test was faster, more sensitive, capable of diagnosis of TB, drug resistance TB and also have overcome the limitation associated with smear and culture with increased accuracy and short turnaround time. Molecular method was also useful to detect the drug resistance related mutation gene simultaneously. Thus, the study aimed to evaluate sensitivity and resistance of linezolid in pre XDR-TB and XDR-TB patient at a tertiary care hospital, Jamnagar, Gujarat, India.

MATERIALS AND METHODS

The Prospective Study conducted during study period of May 2017 to January 2018, received 590 MDR-TB Suspected Sample. This study was conducted in the TB culture and DST laboratory, Department of Microbiology, in our Hospital, From May 2017 to January 2018. There were MDR suspected sputum samples were received in TB culture DST laboratory, Microbiology Department, in our Hospital, during study period. Samples were proceeded to isolate mycobacterium tuberculosis, for evaluation of sensitivity and resistance of linezolid in Pre XDR-TB and XDR-TB at a Tertiary care Hospital, Jamnagar.

There were 2 sputum samples, one spot supervised and one early morning collected in sterile screw cap wide mouth falcon tube and transported from various centers to TB culture-DST laboratory by courier with cold chain maintained. Total 590 MDR suspected sputum samples were received during study period and proceeded for Rapid Detection of Multidrug resistance of Mycobacterium Tuberculosis by direct drug susceptibility testing using the Bactec MGIT 960 system. Patients previously exposed to second line anti-tuberculous treatment, extra pulmonary cases and those not having baseline second-line DST against FQ and AM were excluded. Patients were grouped in to two groups as Linezolid Resistance in Pre XDR-TB and XDR-TB and Linezolid Sensitivity in Pre XDR-TB and XDR-TB. The final data was reported as sensitivity and Resistance of linezolid in Pre XDR and XDR tuberculosis patient with cases of pulmonary MDRTB.

Preparation of sample: All sputum samples were processed following the standard N - acetyline L-Cystein + 2% NaOH (NALC-NaOH) method for digestion, decontamination, and concentration [17, 18]. The concentrated sediment was resuspended in about 2 to 3 mL phosphate buffer (pH 6.8) and mixed thoroughly [17, 18].

Method of Inoculation of MGIT Tube-MGIT tube with specimen number was labelled. The cap was unscrewed and adds 0.5 mL of MGIT OADC. 0.1 mL of reconstituted MGIT PANTA antibiotic mixture was added. Lastly 0.5 mL of concentrated Specimen suspension was added. Also 0.1 mL of suspension on egg -based medium (LJ medium) was added. Tube was incubated at 37°C. Reading of the tubes was done daily starting on the second day of inoculation following the procedure [19].

Detection of positive growth-The instrument signals a tube positive for growth, and an indicator green light shows the exact location of the positive tube. The tube was scanned outside the instrument and observed visually. Mycobacterial growth appears granular and contaminating bacterial growth appears very turbid. Further, the growth of the M. tuberculosis complex was seen and was settled at the bottom of the tube. Positive tube is further confirmed by ZN Stain, Immunochromatography (TB Ag MPT64), growth on egg based medium (LJ medium) and Inoculation on BHI (Brain Heart Infusion Agar plate) [19].

Results/Reporting-The viable mycobacteria present in an inoculated specimen were grow in the MGIT medium and detected visually as well as by fluorescence. Report resulted only when a MGIT tube was positive by the instrument and smear made from the positive broth was also positive for AFB. Negative cultures were reported after completing the incubation protocol of the instrument and visual observation of the negative tubes. Contaminated cultures must be reported as contaminated after confirmation by smear/or subculture

on bacterial medium.

If tube and other confirmatory test give Positive results then drug Susceptibility Testing of sample for diagnosis of MDR-TB, Pre -XDR TB and XDR-TB should be done [19].

Inoculation of positive tube for antibiotics susceptibility testing antibiotic [20,21]- Stock concentration of linezolid drug: 20 mg/10 mL drug solution (India) was prepared in distilled water and after passing through membrane filter; aliquots were made and kept at -70°C. Procedure of positive tube for Drug-If the tube appeared to be positive in day 1 and 2, then left for 5-10 min after mixing well (Vortex) and the supernatant undiluted drug was used for inoculation. Further if the tube was positive in day 3,4 and 5, left for 5-10 min after mixing well (Vortex) and then diluted with 1.0 mL positive broth with 4.0 mL of sterile saline. This was a 1:5 dilution. This could be used further for mixing with diluted culture for inoculation of drug.

Procedure: MGIT tube with specimen number and drug was labelled. Two MGIT Tubes was labelled for culture (1 for Growth control tube without drug and 1 for drug inoculation).0.8 mL of MGIT ODAC was added. 0.1 mL of reconstituted linezolid drug in drug labelled tube was added. 0.5 mL of well mixed culture suspension in to drug containing tube was also added. The same should not to be added to control tube. For Growth control tube, dilute the culture suspension 1:100. Use this diluted suspension to add 0.5 mL in to growth control tube.

Interpretation of DST Results for Second-Line Drug-when Growth Unit (GU) of the GC tube reaches ≥ 400 within the timed protocol, the instrument marks the AST set as complete and interprets the results as S=Susceptible, when the GU of the drug tube is less than 100 and R=Resistant when the GU of the drug tube is 100 or more. Further the errors are described as X200 when system cannot detect sufficient indication of growth in the Growth Control tube in the specified protocol time, and does not provide an interpretation of the AST set results. and as X400 when the system detects indications of possible contaminated or over inoculated tube, and does not provide an interpretation of the AST set results. If DST results for any of the drugs are inconsistent with previous results for the same patient, the results and QC should be reviewed and the test should be repeated. Reporting of DST Results for All Drugs should be recorded as Susceptible (S), Resistant (R) or Test Failed (TF) on the internal lab worksheet/book and the lab requisition form.

RESULTS

We received 590 sputum sample of suspected TB during study period, 468 were culture Positive and 122 were culture Negative. Out of 468 culture positive 294 were MDR-TB, 152 were Pre XDR-TB and 22 were XDR-TB. [Table/Fig-1] shows linezolid resistance and sensitive samples. [Table/Fig-2] shows pre XDR-TB and XDR-TB patients with linezolid resistance and sensitive.

Total culture positive [No. of sample]	Linezolid resistance [No. of sample]	Linezolid sensitive [No. of sample]
468	14	454

[Table/Fig-1]: Linezolid resistance and sensitive.

Category	Linezolid resistance [No. of patient]	Linezolid sensitive [No. of patient]	Total Pre XDR TB [No. of patient]
Pre-XDR-TB	9	143	152
XDR-TB	3	19	22

[Table/Fig-2]: Pre XDR-TB and XDR-TB patient with linezolid resistance and sensitive.

Out of 468 culture positive sputum samples of pulmonary TB, 14 (3%) were Human Immunodeficiency Virus (HIV) infected of which 2 had Pre XDR-TB and 1 had XDR-TB. All 14 were linezolid sensitive. In our study the linezolid sensitivity was higher in Pre XDR-TB and XDR-TB and also in HIV patient it shows higher sensitivity.

DISCUSSION

Present study was done to know the linezolid drug resistance and sensitivity among the Pre XDR-TB and XDR-TB. In present study 468 were culture positive, 14 (3%) were linezolid resistance and 454 (97%) were linezolid sensitive. Present study (Only on linezolid) compared with the Tornheim J et al., Mumbai study was only single drug study (on linezolid) and other study was Irfan Mirza IA et al., study was combination of third line drugs (Linezolid and Meropenem) [Table/Fig-3] [22,23].

Study	Linezolid sensitive (%)	Linezolid resistance (%)
Present Study, 2019	97	3
Tornheim J, Mumbai, 2017 [22]	92	8
Mirza IA et al., Pakistan, 2015 [23]	96	4

[Table/Fig-3]: Comparison of linezolid sensitivity and resistance [22,23].

In Pre XDR/XDR -TB present study results were compared with the Ahmed I et al., study was also combination drugs (Levofloxacin, linezolid and amoxicillin-clavulanate) [24]. So, this study shows that the linezolid is higher sensitivity in the pulmonary tuberculosis patient. Present study linezolid resistance was compared with the Ahmed I et al., for pre XDR-TB and XDR-TB [Table/Fig-4,5] [24].

Study	Linezolid sensitive (%)	Linezolid resistance (%)
Present study, 2019	30%	2%
Ahmed I et al., 2018 [24]	55%	3%

[Table/Fig-4]: Comparison of linezolid sensitivity and resistance in Pre XDR-TB patient.

Study	Linezolid sensitive (%)	Linezolid resistance (%)
Present study, 2019	4%	1%
Ahmed I et al., 2018 [24]	39%	3%

[Table/Fig-5]: Comparison of linezolid sensitivity and resistance in XDR-TB patient.

LIMITATION

As this was a single centre study for short duration (nine months) with sample load of 590 samples, results of present study can't be reflected to the community. Although present study results can provide a base and platform for other researcher and treatment of multidrug resistance tuberculosis but more study are required for the same. Molecular analysis of Mycobacterial Tuberculosis isolates was also not done in present study.

No comparison of linezolid resistance by other different method to do DST are also not available. Patient factors for linezolid resistance was not analysed.

FUTURE RECOMMENDATIONS

In future study should be conducted for longer duration, patient factor like effect, adverse effects, dose regimes etc., and also with follow-up of DR-TB treated with linezolid, and also linezolid drug resistance detected by other molecular methods.

CONCLUSION

Linezolid sensitivity was found high 97% and 3% resistance of linezolid was very low. Such a low level resistance means linezolid can still be the drug of choice to treat complicated MDR/ Pre-XDR and XDR-TB patients with other newer drugs like Bedaquiline/ Delamide as per newer guidelines that will be helpful to achieve TB free India by 2020-2025. Linezolid is effective in treatment of MDR-TB, Pre XDR-TB and XDR-TB patient, but while receiving linezolid, patients should be closely monitored for signs or symptoms of bone marrow toxicity and peripheral and optic neuropathy as many times because of drug adherence issues are seen in patients which can be treated and avoided.

A final comment of present study is that a new drug study is needed for public health, research, replacement of drug with high resistant ratio and support TB programme for TB free India. So, that new drugs are added by TB programmer and gradually understand the role of this drugs in manging to treat TB cases.

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