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'IN VITRO LINEZOLID SUSCEPTIBILITY PROFILE IN DR-TB ISOLATES IN CENTRAL INDIA UTILIZING MGIT 960 LIQUID CULTURE SYSTEM'

Clinical Microbiology

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ABSTRACT

Background & objectives: Linezolid is increasingly being used in drug resistant tuberculosis treatment. This study was aimed to assess Linezolid susceptibility profile in DR-TB isolates at Intermediate Reference laboratory, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh. **Methods:** This was a prospective study conducted from August 2021 to July2022. 110 DR-TB isolates once lab confirmed by Line probe assay were selected and Linezolid Susceptibility testing was done on liquid culture using MGIT 960 Liquid Culture System with defined concentration as per protocol adopted from NTEP. **Results:** Linezolid susceptibility testing was done on 110 DR-TB isolates.3 (2.73%) of the 110 isolates tested for Linezolid susceptibility by MGIT-based susceptibility testing (August 2021 to July 2022) were Linezolid resistant. **Conclusion:** Linezolid resistance poses an important risk to the success of customized treatment regimens. Linezolid susceptibility profile is important to support clinical decision-making for improved patient outcomes.

KEYWORDS

Drug Resistant Tuberculosis, Drug Susceptibility Testing, Linezolid, Mycobacterial Growth Indicator Tube 960.

INTRODUCTION:

Tuberculosis is a curable and preventable disease caused by *Mycobacterium tuberculosis complex.* About a quarter of the world's population has been infected with *M. tuberculosis*.[1] Worldwide, post 2019, Tuberculosis is the 13th leading causes of death and the second leading cause as infectious agent, after COVID-19.[2]

India remains the highest contributor to global TB cases, accounting for 26 % of total cases and 34 % of all deaths worldwide.[2]

Despite the efforts made worldwide to reduce the number of cases of Drug Resistant Tuberculosis, the increasing number of these cases has become a major obstacle in the TB control programmes.[3] Globally, there were an estimated 450, 000 incident cases of MDR-TB or RR - TB (MDR/RR -TB) in 2021, up 3.1% from 437 000 in 2020.[1]

The estimated number of MDR/RR-TB cases in India is 124000 (9.1/lakh population).[4] The estimated number of MDR and XDR-TB (Extensively Drug Resistant Tuberculosis) cases to have been put on treatment as per the global TB report 2021 was 4 per 100,000 and 1 per 100,000 population, respectively.[5]

In order to prevent treatment failure for MDR-TB cases and also to halt the progression of MDR-TB cases to XDR-TB which is not only difficult but also very expensive to treat, there is urgent need to device strategies for rapid and early detection of pre- XDR TB (pre-Extensively Drug Resistant Tuberculosis).

For the control and management of the rising burden of DR-TB universal access to drug susceptibility testing (DST) and individualized treatment approaches have been initiated under NTEP.[24] The solid medium based DST methods are slow, requiring readings at 4 to 6 weeks, which delays the detection of drug resistance and risks spread of the drug resistant strains. This has led to the development of newer DST methodologies, including liquid culture systems and molecular line probe assays which have also received recommendations from WHO. [6,7]

Linezolid is being recommended for treatment of MRSA (Methicillin Resistant *Staphylococcus aureus*) by various technical bodies also the prevalence of MRSA varies in different regions with 13-74% prevalence worldwide.[27] In India overall prevalence of MRSA among *Staphylococcus aureus* isolates in 2020 was 41.6% (26.8% - 74.7%). [28] Recently Linezolid has been introduced in group-A of second-line anti-TB drugs.[8] Linezolid is an oxazolidinone antibiotic that acts by inhibiting bacterial protein synthesis by preventing the fusion of 30S and 50S ribosomal subunits. [9] It readily gets distributed to well-perfused regions of the body, penetrates well into bronchoalveolar tissue and acts by inhibiting protein synthesis at an early stage of translation. The predominant molecular mechanisms associated with Linezolid resistance in *Mycobacterium tuberculosis* are mutations in ribosomal genes 23S rRNA (encoding ribosomal RNA, *rrl*) and *rplC* (encoding the L3 protein).[26] The drug itself has been in regular use for treatment of non-mycobacterial bacterial infections hence the probability of acquiring antimicrobial resistance is high in dormant *Mycobacterium tuberculosis* strains and liable for horizontal transfer due to sustained antimicrobial pressure, especially in high burden countries.

Potential risk factors for Linezolid resistance have been identified: (*i*) Linezolid dosing is often reduced because of associated mitochondrial toxicity, leading to suboptimal exposure for efficacy and resistance suppression and to the selection of resistant mutants and (*ii*) Linezolid may be added to a failing or inadequate regimen, exacerbating the risk of acquired resistance.[10,11]

Linezolid has been considered highly effective and strongly recommended for inclusion in all regimens for treatment of MDR-TB and XDR-TB unless contraindicated. We do need regional data of Linezolid resistance so that recommendations to use Linezolid for treatment of DR-TB can be implemented at the regional level under the policies of NTEP. Our study was aimed to assess Linezolid susceptibility profile in DR-TB isolates.

MATERIAL AND METHODS:

This prospective study was undertaken at the Intermediate Reference Laboratory, Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, India over a period of 1 year from August 2021 to July 2022.The study was approved by the institutional ethics committee of the hospital. 110 DR-TB isolates were subjected to Mycobacterial Growth Indicator Tube -960 (MGIT -960) liquid culture Linezolid susceptibility testing.

Exclusion criteria

1. All first line sensitive cases which were not under resistant treatment protocol.
2. Contamination
3. Non-Tuberculous Mycobacteria (NTM)

Once lab confirmed by Line probe assay, DR-TB isolates from MGIT-960 liquid culture were selected and liquid culture Linezolid susceptibility testing *Mycobacterium tuberculosis* was done in BACTEC MGIT-960 TB System, isolates were preserved for future DST. Whole procedure including drug reconstitution, liquid culture drug susceptibility testing was performed in a Class II-B1 Biosafety cabinet in BSL-3 lab with adherence to the Standard operating procedure.[12,13,14] Critical concentrations (µg/ml) for DST by BACTEC MGIT-960 liquid culture for Linezolid (Sigma-Aldrich) was 1 µg/ml. M*ycobacterium tuberculosis* strain *H37Rv* was used as reference control strain and run along with each batch. The instrument monitored the entered susceptibility test set. Once the test was complete (within 7 to 21 days), the instrument indicated that the results were ready. The susceptibility Set Carrier were scanned and print was taken. The instrument printout indicated susceptibility results for each drug. Results were qualitative: Susceptible (**S**), Resistant (**R**) or Indeterminate (**X**).

RESULTS:

110 DR-TB isolates from 17 districts were included in our study and all were subjected to drug susceptibility testing for Linezolid on liquid culture. In our study Linezolid was found to be susceptible in 107 (97.27%) isolates and resistant in 3 (2.73%) isolates as shown in figure no.1.

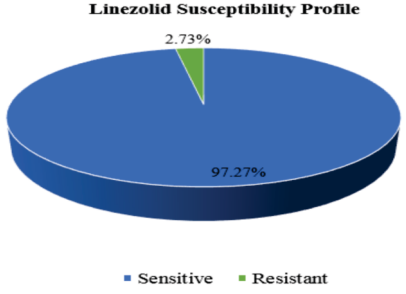

Figure. No.1- Pie chart showing Linezolid susceptibility profile by Second Line-Liquid Culture Drug Susceptibility Testing

As shown in table no.1, in our study DR-TB isolates were 38 (34.55%) MDR, 61 (55.45%) Pre-XDR and 6 (5.45%) XDR 2 (1.82%) each were Isoniazid mono resistant and Rifampicin mono resistant. Also 1 isolate was Rifampicin resistant along with Fluoroquinolone resistant. On Linezolid susceptibility testing Linezolid resistance was seen in 1 MDR TB & 2 pre-XDR TB isolates.

Table No.1- Linezolid Susceptibility profile by Second Line-Liquid Culture Drug Susceptibility Testing in types of DR-TB

Type of DR TB	Linezolid Susceptibility Profile			
Type of DR TB	**Number**	**Percentage**	**Sensitive**	**Resistant**
Isoniazid mono resistant	2	1.82%	2	0
Rifampicin mono resistant	2	1.82%	2	0
Rifampicin-Fluoroquinolone(Rif-FLQ) resistant	1	0.91%	1	0
MDR	38	34.55%	37	1
Pre-XDR	61	55.45%	59	2
XDR	6	5.45%	6	0

Table No.2 - Regional distribution of Linezolid susceptibility profile of 110 isolates belonging to DR-TB patients from 17 districts

District	Number of patients	Percentage	Linezolid Sensitive	Linezolid Resistant
Agar	1	0.91%	1	0
Alirajpur	1	0.91%	1	0
Barwani	3	2.73%	3	0
Bhind	1	0.91%	1	0
Burhanpur	3	2.73%	3	0
Dewas	7	6.36%	7	0
Dhar	8	7.27%	8	0
Hoshangabad	1	0.91%	1	0
Indore	27	24.55%	27	0
Jhabua	13	11.82%	12	1
Khargone	2	1.81%	2	0
Mandsaur	4	3.64%	4	0
Neemuch	1	0.91%	1	0
Raisen	2	1.81%	2	0
Rajgarh	8	7.27%	8	0
Ratlam	25	22.73%	23	2
Ujjain	3	2.73%	3	0

110 isolates were from patients (61new and 49 follow up DR-TB cases) from 17 districts of central India, Madhya Pradesh, 27(24.55%) were from Indore, 25(22.73%) from Ratlam,13(11.82%) from Jhabua as mentioned in table no.2. patients. Of the 3 isolates resistant to Linezolid, 2 belonged to patients from Ratlam (1 MDR and 1 pre-XDR) and 1 patient from Jhabua district (pre-XDR).

DISCUSSION:

Present study titled 'In Vitro Linezolid Susceptibility profile in DR-TB isolates in Central India utilizing MGIT- 960 Liquid Culture System' was conducted in Intermediate Reference Laboratory (IRL), Department of Microbiology M.G.M. Medical College, Indore (M.P.).

Out of 110 DR-TB isolates included in our study, 2 (1.82%) each were Isoniazid mono resistant and Rifampicin mono resistant, MDR were 38(34.55%), in contrast to a study done by Polu GP et al., 2019 in Andhra Pradesh in which INH mono resistant were15.13%, RIF mono resistant 3.59%, and 12.54% were multi drug resistant.[15] In our study pre-XDR and XDR were 61 (55.45%) and 6(5.45%) respectively. In a study by Singhal P et al., 2016 conducted in North India MDR, pre-XDR, XDR were 39.7%, 49.4 % and 11.4%, respectively.[16]

Of 110 DR-TB isolates, 3 (2.73%) were found to be Linezolid resistant and 107 (97.27%) were Linezolid sensitive similar to study done in Gujarat by Dipali et al., 2019 in which 3% resistance was seen, another study done in Mumbai by Nambiar et al., 2021 in which 3.1% resistance was seen.[10,17] While other studies have comparatively reported Linezolid resistance rates as high as 30% in study done South Africa by Sean Wassermann et al.,2022 [18], 5.6% in study by Pang et al., 2017 done in Beijing [19] and 5.9% in study by Ahmed et al., 2013 Karachi.[20]

In our study Linezolid resistance was found in 1 out of 38 MDR TB and 2 out of 61 pre-XDR isolates. A systematic review and meta-analysis to investigate the prevalence of Linezolid resistance among MDR-TB isolates done by Azimi et al., [21] from 1 January 2000 to 12 April 2021, revealed that the overall estimate of Linezolid resistance among clinical isolates of MDR-TB was 4.2%. As Linezolid has been included in group A priority drug for DR-TB treatment [8,22,23], any percentage of resistance to Linezolid is matter of concern which needs to be addressed.

Total patients from Ratlam district were 25 and as seen in this study 2 of them were found to be resistant to Linezolid which comes to be 8% resistant to Linezolid in that region which is significant and alarming. Jhabua district is notified tribal district of Central India thus it is prudent to keep the track of recommencing antitubercular drug resistance in this district.

CONCLUSION:

One of the cornerstones for successful management of drug resistant tuberculosis is customized individualized treatment protocol with the help of Universal Drug Susceptibility Testing (U-DST) under purview of current NTEP programme.[24] Timely access to accurate DST is essential for every TB patient to inform and guide the therapeutic decision.[25]Adjusting the treatment according to the in-vitro susceptibility profile of Linezolid may be helpful to optimize the treatment in MDR/XDR *Mycobacterium tuberculosis*.

Linezolid has been included in drug regimens for DR-TB patients as per WHO guidelines. Increased use of Linezolid has not been associated with increased global DST capacity for Linezolid, so many new prescriptions will occur without confirmation of Linezolid susceptibility. This is important, considering the increasing recognition of circulating Linezolid resistance. To maximize treatment benefits while minimizing toxicity, DST remains an important tool to identify Linezolid resistance.

Linezolid though being schedule H1 drug, its use is still unchecked, also it has not been covered for monitoring under an Over-the-counter

sell record keeping similar to other antitubercular drugs. At tertiary level centres or in big cities, the use of Linezolid is quite rampant for the treatment of community acquired Pneumonia, skin and soft tissue infections and other infections caused by Gram-positive bacteria including MRSA/VRE which in turn has increased the chances of emergence of Linezolid resistant *Mycobacterium tuberculosis* strains. There should be adoption of a Linezolid restriction policy in India and also efforts to create awareness among practising doctors to use Linezolid cautiously. Our antimicrobial armamentarium should be used appropriately for benefit of future generations.

Conflicts Of Interest: None

Ethics Clearance:
This study was started after ethics committee clearance from institution on 27th August 2021 from the Ethics and Scientific review committee M.G.M. Medical College & M.Y.H. Indore (M.P.)

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