Iranian Journal of Microbiology

Volume 17 Number 6 (December 2025) 936-941
DOI: http://doi.org/10.18502/ijm.v17i6.20361

ORIGINAL ARTICLE

Estimation of second line anti-tubercular drug susceptibility to *Mycobacterium tuberculosis* in clinical isolates

Harisree Sudersanan[1*], Nikhilesh Menon Ravikumar[2], Baijayanti Mishra[3]

[1]Department of Microbiology, Government Medical College, Ernakulam, Kerala, India
[2]Department of Health Services, Assistant Surgeon, State Nodal Officer (Lab Network), Govt of Kerala, India
[3]Department of Microbiology, St. John's Medical College, Bangalore, India

Received: December 2024**, Accepted:** November 2025

ABSTRACT

Background and Objectives: *Tuberculosis* (TB) is an infectious disease that is among the most common in the world and is a leading cause of high mortality and morbidity. In India, there is very limited data on second line drug susceptibility testing. The aim of the study was to find out the prevalence of Multi Drug Resistant (MDR) isolates among *Mycobacterium tuberculosis* (MTB) complex strains and to assess the sensitivity pattern to four commonly used 2nd line anti-tubercular drugs irrespective of their MDR status.

Materials and Methods: A 61 culture-positive strains of the tuberculosis complex (smear positive or negative) in Mycobacterium Growth Indicator Tube (MGIT) and Lowenstein Jensen (LJ) from various clinical samples were included. We performed MGIT 1st and 2nd line susceptibility testing for tuberculosis.

Results: Among the 61 isolates, 12 (19.6%) were multi drug resistant. Capreomycin resistance was observed in 17 (27.8%) isolates, kanamycin resistance in 30 (49.1%), ofloxacin resistance in 5 (8.1%), and ethionamide resistance in 6 (9.8%) isolates. Resistance to kanamycin and ethionamide was more common among patients with multi drug resistant tuberculosis (MDR-TB) than among those with non-MDR-TB.

Conclusion: The MGIT system has surpassed solid culture and is an excellent method for performing culture and drug sensitivity testing for tuberculosis. However, its use remains limited by economic and logistical challenges. The high prevalence of aminoglycoside resistance suggests the need to preserve these drugs for treating patients with MDR-TB.

Keywords: *Mycobacterium tuberculosis*; Infections; Antitubercular drugs; Resistant

INTRODUCTION

Tuberculosis TB is one of the most common infectious diseases globally and is a major cause of high mortality and morbidity (1). A major aim of the DOTS strategy is to prevent the development of Multidrug Resistant Tuberculosis (MDR- TB), which is defined as resistance to both isoniazid and rifampicin and is more difficult to cure. In fact, it requires a long treatment. Further, this treatment is with expensive and often toxic multidrug regimens (2). According to estimates given by WHO, there were 9.6 million new TB cases and 4,80,000 new cases of MDR-TB in 2015. Most of these occurred in Asia and Africa.

*Corresponding author: Harisree Sudersanan, MD, Department of Microbiology, Government Medical College, Ernakulam, Kerala, India.
Tel: +91-9447787057 Fax: +91-8025530070 Email: ganashree2u@gmail.com

In 2015, the approximate number of people who died due to TB was 1.4 million. In 2006, WHO gave a definition of Extensively Drug Resistant Tuberculosis (XDR-TB). XDR-TB is a more dangerous strain of *Mycobacterium tuberculosis* which is resistant to isoniazid, rifampicin, at least one second-line injectable drug and one fluoroquinolone (3). In 2021, WHO revised the definition of XDR-TB. It now states that XDR-TB is TB caused by *M. tuberculosis* strains that meet the definition of MDR/RR-TB, but it is also resistant to any fluoroquinolone and at least one additional Group A drug. Group A drugs, which include levofloxacin, moxifloxacin, bedaquiline, and linezolid, are the most potent group of second-line medicines. They are core agents in the longer treatment regimens used for drug-resistant TB (4).

According to the Global TB Report 2021, the estimated incidence of TB in India for 2020 was 188 per 100,000 population (uncertainty interval: 129-257). In 2021, the number of incident TB patients notified (new & relapse) was 19,33,381, which was 19% higher than the 16,28,161 cases notified in 2020. In 2020, the mortality rate for all types of tuberculosis was about 37 per 100,000 people (34-40 per 100,000 people). According to the Global TB Report 2021, an estimated 4 people per 100,000 population were started on treatment for MDR-TB, compared to 1 per 100,000 for XDR-TB (5).

The treatment for XDR-TB is more complicated and requires the availability of second line anti-tuberculosis drug and the related antimicrobial susceptibility testing. Empirical treatment for tuberculosis is usually practiced before susceptibility testing results are available. Reducing mortality, morbidity, and transmission are the benefits of knowing the results before treatment. Knowing the local susceptibility pattern is immensely helpful for empirical treatment in MDR and XDR-TB (3). In the past, drug susceptibility testing was performed on specimens cultured on solid media, such as Lowenstein-Jensen (LJ) media. This method is labour intensive, less sensitive and more time consuming when compared to the newer liquid culture method, MGIT (Mycobacterial Growth Indicator Tube) (6). In, India, data regarding second line drug susceptibility testing is very limited (2). In this study we aim to determine the prevalence of MDR isolates among strains of MTB complex by using MGIT and to evaluate the sensitivity pattern to the four commonly used 2nd line anti-tubercular drugs irrespective of their MDR status.

MATERIALS AND METHODS

This study was conducted in a 2000 bedded tertiary care hospital at Bangalore, India. The study took place at microbiology department after obtaining clearance from the ethical committee. This hospital has been using the MGIT since 2012, for culture and first-line susceptibility testing for MTB. The study included 61 culture-positive (smear-positive/negative) isolates of the *Mycobacterium tuberculosis* complex, identified by both MGIT and LJ culture, from various clinical samples. Second line susceptibility testing for tuberculosis was initiated at our hospital and was a part of the study.

Inclusion criteria. All 61 culture-positive *Mycobacterium tuberculosis* complex isolates as well as both pulmonary and extrapulmonary specimens were included.

Exclusion criteria. Specimens that were smear-positive or PCR-positive but culture-negative were excluded from the study.

Study design. Descriptive study.

Ziehl Neelsen staining and culture by MGIT and LJ were done on 418 samples. Culture was done after the NALC method of decontamination. Samples that yielded growth on LJ or MGIT were confirmed to be MTB complex by MPT 64 card test and were then subjected to first-line and second-line drug sensitivity testing.

Drug susceptibility testing. The strains isolated and identified as *Mycobacterium tuberculosis* were subjected to drug susceptibility testing by Manual Micro-MGIT (Becton Dickinson Microbiology System, Sparks, NV, USA). The raw powder for capreomycin, ethionamide, ofloxacin, and kanamycin was obtained from Sigma-Aldrich (USA). Lyophilized drugs were obtained from BD (Becton, Dickinson and Company, USA). The critical concentrations used for first- and second-line drug susceptibility testing were as follows: rifampicin (1 μg/mL), isoniazid (0.1 μg/mL), capreomycin (2.5 μg/mL), ethionamide (5 μg/mL), kanamycin (2.5 μg/mL), and ofloxacin (2 μg/mL).

Procedure. All drugs were obtained in chemically pure powder, which was weighed and dissolved as follows: kanamycin and capreomycin in sterile distilled water, ofloxacin in 0.1N NaOH, and ethio-

namide in dimethyl sulfoxide. We prepared a stock solution of 1000µg/ml and stored it at -20°C for no more than 6 months. A final stock solution of 2.5 µg/ml was prepared. Then, 200 µl of this stock solution was aliquoted into small sterile containers and was stored at -20°C. The other stock solutions were stored at -70°C with a maximum shelf life of six months.

The lyophilized drugs (BD) for rifampicin, isoniazid, ofloxacin and capreomycin were reconstituted as per the manufacturer's instructions by addition of 4 ml of sterile distilled water.

After isolation and identification, the strains were subjected to drug susceptibility testing by MGIT (according to the manufacturer's instructions). 100 µl of the respective drug was added to the MGIT tubes for drug susceptibility testing, along with a growth control tube without drug which was taken as control. Each batch of the new drug was subjected to quality control tests using the H37Rv (ATCC 27294) strain. If this strain was resistant to any, all tests of that batch were repeated.

RESULTS

Among the 61 culture-positive samples, the breakdown was as follows: 33 were pulmonary (18 bronchoalveolar lavage, 14 sputum and one gastric aspirate) and 28 were extrapulmonary samples (including pus, tissue, ascitic fluid, pleural fluid, bone marrow, CSF). Of the isolates, 38 (62.3%) were from male patients and 23 (37.7%) were from female patients. The majority of the positive cases were found in 20-30 and >50-year age groups, as shown in Fig. 1.

Comparison of direct microscopy with culture. Among the 61 culture-positive samples, 13 (21.3%) were negative by smear microscopy. Of these smear-negative samples, 11 were extrapulmonary specimens (pus, tissue, CSF and bone marrow), and the remaining two were pulmonary specimens.

Comparison of GeneXpert and culture. Among the culture positive samples, GeneXpert was done for 23 samples (11 pulmonary, 12 extrapulmonary). Three samples (tissue, pus, sputum) gave discordant results by GeneXpert when compared to culture. Among the three samples, the tissue and sputum samples were smear-negative, while the pus specimen was smear-positive. Further analysis of the discordant results is shown in Table 1.

Among the 61 isolates, 12 (19.6%) were found to be multi drug resistant. Isoniazid monoresistance was seen in 10 (16.4%) samples while rifampicin monoresistance was not observed in any of the samples. Second line drug susceptibility among the 61 isolates is shown in Table 2.

Among the 12 MDR strains, resistance to capreomycin, kanamycin, ofloxacin, and ethionamide was observed in 6 (50%), 9 (75%), 2 (16.7%), and 4 (33.3%) isolates, respectively. The drug susceptibility testing results for capreomycin and ofloxacin showed 100% correlation between the powder and lyophilized drug formulations. The difference in drug resistance patterns between MDR and non-MDR-TB is shown in Table 3. The difference in drug resistance patterns between XDR and non-XDR-TB is shown in Table 4. Resistance to multiple second-line drugs among MDR TB patients is shown in Table 5.

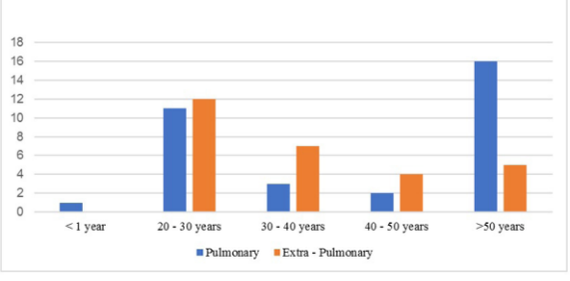

Fig. 1. Age wise distribution of the patients

Table 1. Analysis of discordant results (culture positive and GeneXpert negative)

GeneXprt ID	Sample	AFB smear	Possible reasons for discordance
GX 12	Tissue	-	Blood stained
GX28	Sputum	-	Not Known. Sample was repeated with additional processing steps and still negative
GX54	Pus	+	Blood stained

Table 2. Capreomycin, ofloxacin, kanamycin and ethionamide sensitivity pattern

Drug	Resistant	Sensitive	Monoresistance
Capreomycin	17 (27.8%)	44 (72.1%)	1 (1.63%)
Ofloxacin	5 (8.1%)	56 (91.8%)	2 (3.27%)
Kanamycin	30 (49.1%)	31 (50.81%)	11 (18.03%)
Ethionamide	6 (9.83%)	55 (90.1%)	nil

Table 3. Comparison of drug resistance percentages between non MDR -Mtb and MDR -Mtb

Drugs	Non MDR Mtb N=49	MDR Mtb N=12	P value
Fluroquinolones: Ofloxacin	3	2	0.23
Injectable antitubercular drugs			
Capreomycin	11	6	0.09
Kanamycin	21	9	0.04
Other second line Antitubercular Drug			
Ethionamide	2	4	0.002

Table 4. Comparison of drug resistance percentages between non-XDR Mtbs and XDR -Mtbs among MDR isolates

Drugs	Non XDR Mtb N=10	XDR Mtb N=2	P value
Fluroquinolones: Ofloxacin	0	2	
Injectable antitubercular drugs			
Capreomycin	5	1	1.000
kanamycin	7	2	0.4
Other second line Antitubercular Drug			
Ethionamide	3	1	0.6

Table 5. Percentage of resistance to multiple second line drugs among MDR-TB patients

Drugs	N (12)	%
Two drugs		
CAP + KM	6	50
CAP + ETH	4	33.3
Three Drugs		
CAP + ETH +KM	4	33.3
XDR TB		
MDR+KM+OFLX+_ETH	2	16.7

MDR TB- Multi drug Resistant Tuberculosis, KM- kanamycin, CAP- capreomycin, ETH- Ethionamide, OFLX- Ofloxacin

DISCUSSION

This study was performed to assess second-line drug susceptibility among patients with tuberculosis. Rapid and accurate second-line drug susceptibility testing is extremely important for the timely initiation of an appropriate antituberculosis regimen. The rising number of drug-resistant strains of MTB has accelerated efforts to develop more rapid and accurate methods for susceptibility testing (7). The MGIT is one of the most reliable methods for susceptibility testing; its lack of radioactivity, noninvasive nature, and labor-saving design make it a better option than the BACTEC 460 TB system. In this method , a strain is defined as resistant if relative growth in the drug containing tube equals or exceeds that in the growth control tube (GC) and susceptible ,if the relative growth is less than the growth in GC tube (8).

In this study, the prevalence of MDR strains among the positive samples was 19.6%. This finding is concordant with a study conducted in India by Lohiya et al., which reported an MDR-TB prevalence of 26.7% (9). According to WHO data from 2021, the vast majority of TB cases were from eight countries, which included India (28%), Indonesia (9.2%), China (7.4%), the Philippines (7%), Pakistan (5.8%), Nigeria (4.4%), Bangladesh (3.6%), and the Democratic Republic of Congo (2.9%) (10).

During the course of the study, second line susceptibility testing was done for all the positive samples irrespective of their resistance status. Among the four second-line drugs tested, ofloxacin and kanamycin resistance was seen in 16.7% and 75% of the MDR isolates respectively. Ofloxacin resistance among MDR isolates in other studies varied, with reported rates of 0% in Tanzania (11), 30.2% in Taiwan (3), and 41.5 % in China (12). Ofloxacin resistance in our study (16.7%) was concordant with another study conducted in India by ParamaShivan et al. (2), which showed an ofloxacin resistance of 16.4% among MDR isolates. Our study showed a surprisingly high prevalence of kanamycin resistance among both MDR and non-MDR isolates, amounting to 75% and 42.9%, respectively. This finding is discordant with other studies, which reported relatively low kanamycin resistance, ranging from 5% to 17% (11, 12). The detection of resistance to either ofloxacin or kanamycin is highly significant, as suggested by Mlambo et al., because patients with such resistance are at high risk of developing XDR-TB if not managed appropriately (13). Kanamycin resistance was significantly more common among patients with MDR-TB than among those with non-MDR-TB ($p < 0.05$).

Similar to kanamycin, capreomycin resistance among MDR isolates was also high, at 50%. This finding was discordant with the study conducted in China, which showed that 20.3% of the MDR isolates were resistant to capreomycin (12). The detection of

capreomycin resistance is immensely significant because it is associated with early mortality in XDR-TB patients. Although capreomycin resistance was very high, there was no difference in its resistance pattern between MDR and non-MDR-TB patients. The prevalence of ethionamide resistance among MDR isolates in our study (33.3%) was similar to the 30.2% reported by Kuo et al. (3). Ethionamide resistance was significantly more common among patients with MDR-TB than among those with non-MDR-TB ($p < 0.05$).

Although our study was based on samples that were culture-positive by MGIT, GeneXpert was also performed on a subset of 23 of these culture-positive samples. Three samples yielded discordant (negative) results compared to culture. Among the three discordant results, two samples were blood-stained. Other studies have explained the limitations associated with PCR when biological inhibitors, such as blood, are present in samples (14). In their study, Khan et al. noted that false negatives in GeneXpert can be reduced by using an extended processing step (15). However, in our study, the GenXpert results of the blood-stained samples remained negative even after an extended processing step.

CONCLUSION

To the best of our knowledge, this is one of the very few studies from southern India reporting on second-line drug susceptibility for tuberculosis. Among the 61 isolates, 12 (19.6%) were found to be multi drug resistant. Aminoglycoside resistance was found to be very high in our study when compared to other published studies. Kanamycin and ethionamide resistance were more commonly observed among MDR-TB patients than among non-MDR-TB patients. The uptake of second-line DST remains limited by economic and logistical challenges. Second-line DST costs more than other TB diagnostics and requires specialized laboratory facilities, expertise, and infrastructure to transport specimens and relay results (16).

ACKNOWLEDGEMENTS

Author would like to thank all the staff at St Johns Medical College, Bangalore for their support throughout the study.

REFERENCES

1. Zhao P, Fang F, Yu Q, Guo J, Zhang J-h, Qu J, et al. Evaluation of BACTEC MGIT 960 system for testing Susceptibility of *Mycobacterium tuberculosis* to First-Line drugs in China. *PLoS One* 2014; 9(9): e99659.
2. Paramasivan CN, Rehman F, Wares F, Sundar Mohan N, Sundar S, Devi S, et al. First- and second-line drug resistance patterns among previously treated tuberculosis patients in India. *Int J Tuberc Lung Dis* 2010; 14: 243-246.
3. Kuo CY, Wang WH, Huang CH, Chen YH, Lu PL. Resistance to first- and second-line antituberculosis drugs in southern Taiwan: Implications for empirical treatment. *J Microbiol Immunol Infect* 2018; 51: 88-93.
4. Viney K, Linh NN, Gegia M, Zignol M, Glaziou P, Ismail N, et al. New definitions of pre-extensively and extensively drug-resistant tuberculosis: update from the World Health Organization. *Eur Respir J* 2021; 57: 2100361.
5. Central TB Division. INDIA TB REPORT 2022 Coming Together to End TB Altogether. Central TB Division, Ministry of Health and Family Welfare. (2022).
6. Lawson L, Emenyonu N, Abdurrahman ST, Lawson JO, Uzoewulu GN, Sogaolu OM, et al. Comparison of Mycobacterium tuberculosis drug susceptibility using solid and liquid culture in Nigeria. *BMC Res Notes* 2013; 6: 215.
7. Woods GL. Susceptibility testing for mycobacteria. *Clin Infect Dis* 2000; 31: 1209-1215.
8. Ardito F, Posteraro B, Sanguinetti M, Zanetti S, Fadda G. Evaluation of BACTEC Mycobacteria Growth Indicator Tube (MGIT 960) automated system for drug susceptibility testing of *Mycobacterium tuberculosis. J Clin Microbiol* 2001; 39: 4440-4444.
9. Lohiya A, Suliankatchi Abdulkader R, Rath RS, Jacob O, Chinnakali P, Goel AD, et al. Prevalence and patterns of drug resistant pulmonary tuberculosis in India-A systematic review and meta-analysis. *J Glob Antimicrob Resist* 2020; 22: 308-316.
10. WHO (2021). Global tuberculosis report. World Health Organization, Geneva.
11. Mpagama SG, Houpt ER, Stroup S, Kumburu H, Gratz J, Kibiki GS, et al. Application of quantitative second-line drug susceptibility testing at a multidrug-resistant tuberculosis hospital in Tanzania. *BMC Infect Dis* 2013; 13: 432.
12. Chen Y, Yuan Z, Shen X, Wu J, Wu Z, Xu B. Resistance to Second-Line antituberculosis drugs and delay in drug susceptibility testing among multidrug-resistant

tuberculosis patients in shanghai. *Biomed Res Int* 2016; 2016: 2628913.
13. Mlambo CK, Warren RM, Poswa X, Victor TC, Duse AG, Marais E. Genotypic diversity of extensively drug-resistant tuberculosis (XDR-TB) in South Africa. *Int J Tuberc Lung Dis* 2008; 12: 99-104.
14. Al-Soud WA, Jönsson LJ, Rådström P. Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. *J Clin Microbiol* 2000; 38: 345-350.
15. Bhardwaj A, Khan S, Kumar A, George L, Mehta A, Radhakrishnan K. Assessing the Utility of GeneXpert MTB/Rif Assay in a Tertiary Care Centre in Southern India with Established Microscopy and Liquid Culture Facilities. *J Assoc Physicians India* 2019; 67: 31-34.
16. Kendall EA, Cohen T, Mitnick CD, Dowdy DW. Second line drug susceptibility testing to inform the treatment of rifampin-resistant tuberculosis: a quantitative perspective. *Int J Infect Dis* 2017; 56: 185-189.