International Journal of Advances in Medicine
Kumar R et al. Int J Adv Med. 2019 Dec;6(6):1918-1923
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: http://dx.doi.org/10.18203/2349-3933.ijam20195251

Original Research Article

Drug resistance profile among multi-drug resistant tuberculosis patients at diagnosis and correlation with history of anti-tubercular treatment in a tertiary care center

Rahul Kumar[1], Rajiv Garg[2], Silpa Kshetrimayum[3]*, Amita Jain[4]

[1]Department of Respiratory Medicine, All India Institute of Medical Sciences, Patna, Bihar, India
[2]Department of Respiratory Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India
[3]Department of Respiratory Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, India
[4]Department of Microbiology, King George's Medical University, Lucknow, Uttar Pradesh, India

Received: 04 October 2019
Revised: 05 November 2019
Accepted: 12 November 2019

***Correspondence:**
Dr. Silpa Kshetrimayum,
E-mail: ksh.silpa@gmail.com

ABSTRACT

Background: Drug Resistant Tuberculosis (DR-TB) is a major threat to the realization of the goal of a TB free world in the near future. It is important to study the reasons for the increasing number of such cases so that effective action can be taken to control this growing epidemic.
Methods: Sputum from 36 patients diagnosed with acquired pulmonary Multidrug Resistant Tuberculosis (MDR-TB) were subjected to first- and second-line Drug Sensitivity Testing (DST) after liquid culture in mycobacterium growth Indicator Tube (MGIT). Primary MDR-TB cases were excluded. The relation of the drug sensitivity profile with the history of prior treatment taken was statistically analysed.
Results: Majority of the patients had received appropriate treatment, and most had adhered to prescribed treatment. Among the 36 patients, 24(66.7%) were found to be Pre-Extensively Drug Resistant (Pre-XDR-TB) and 4(11.1%) were extensively drug resistant XDR-TB cases. Inappropriate prescription of fluoroquinolone (FQ) was found to be most common. Prior intake of any drug was not found to significantly affect subsequent resistance to that drug.
Conclusions: Fluoroquinolone resistance is quite common in patients with DR-TB (66.7%). This study did not find the prior use of FQ or any other drug to significantly affect subsequent resistance to the drug. Primary drug resistance is thus a major concern. 11.1% patients were found to be XDR-TB cases. Hence DST for first- and second-line drugs should be done at the time of diagnosis to avoid failure of treatment with a predesigned regimen.

Keywords: Drug resistant tuberculosis, Drug sensitivity testing, Extensively drug resistant, Fluoroquinolone, Multidrug resistant, Multidrug resistant

INTRODUCTION

Drug Resistant Tuberculosis (DR-TB) is the biggest challenge to the achievement of the goals of the end TB strategy of the World Health Organization (WHO). Despite continuous evidence-based policy recommendations on the treatment and care of such patients it continues to hamper the efforts to reduce tuberculosis burden worldwide1. Drug resistance can be classified into Multidrug Resistant (MDR) where the *Mycobacterium bacilli* develop resistance to both rifampicin and isoniazid or rifampicin alone, Pre-Extensively Drug Resistant (Pre-XDR) where the MDR strain is additionally resistant to a fluoroquinolone or one

of the three injectable aminoglycosides (Amikacin, Kanamycin, Capreomycin) and Extensively Drug Resistant (XDR) where the bacilli is resistant to rifampicin with or without isoniazid, a fluoroquinolone and at least one of the three injectable aminoglycoside. Polydrug resistant strains are those that are resistant to more than one first line drug other than both rifampicin and isoniazid together. These groups of patients are more difficult to treat than drug susceptible Tuberculosis (TB) with treatment success rates of 50-60% only for MDR cases.[1] According to the global tuberculosis report 2018, an estimated 558,000 new cases of rifampicin resistant TB were reported in 2017 of which the majority were from India (24%), followed by China (13%) and the Russian Federation (10%).[2]

The DR-TB population is heterogenous, comprising of patients who have never been exposed to first- or second-line Anti-Tuberculosis Treatment (ATT) and those who have had treatment failure or retreatment.[3] Factors contributing to resistance often include poor adherence of patients to ATT, inappropriate treatment regimen, inadequate dosage and duration of treatment and non-compliance to national guidelines and standards of TB care by clinicians.[4] Widespread use of fluoroquinolones and aminoglycosides to treat other bacterial infections may also contribute to evolution of resistance to these agents.[5]

There is limited data on the drug resistance profile and prevalence of Pre-XDR and XDR-TB among acquired MDR-TB cases. The present study explores the drug resistance profile among acquired MDR-TB isolates and its correlation with history of ATT in the form of drugs previously taken, adherence to and appropriateness of the regimen, all of which contribute to the development of resistance.

Aims and objectives of the study was to study the drug resistance profile among drug resistant pulmonary tuberculosis patients, the correlation of previous intake of a drug with resistance to that drug, factors contributing to resistance.

METHODS

Department of Respiratory Medicine, King George's Medical University, Lucknow.

Inclusion criteria

- Newly diagnosed pulmonary MDR-TB cases who had received prior treatment for tuberculosis for at least 1 month and had complete record of treatment.

Exclusion criteria

- Cases of extrapulmonary tuberculosis
- Primary MDR-TB cases
- Contacts of known MDR or XDR-TB
- Patients without proper record of prior treatment for tuberculosis.

It was a single center, prospective, observational study from August 2016 to July 2017. A total of 36 patients diagnosed to be pulmonary MDR-TB cases via the Line Probe Assay (LPA) technique and who fulfilled the inclusion criteria were included in the study. A detailed history of symptoms and treatment received were obtained from the patient and his old records. 3-5 ml of good quality sputum from the patient was sent for liquid culture in Mycobacterium Growth Indicator Tube (MGIT). The isolates were subjected to first- and second-line Drug Sensitivity Test (DST). The critical concentration of drugs used were 1 µg/ml for rifampicin, 0.1 µg/ml for isoniazid, 5 µg/ml for ethambutol, 1 µg/ml for streptomycin, 2 µg/ml for ofloxacin, 1 µg/ml for amikacin, 2.5 µg/ml for kanamycin and 2.5 µg/ml for capreomycin. For each isolate, a Growth Control (GC) tube with growth supplement but without drug was used. The relative growth ratio between the drug containing tube and drug free GC tube was generated by the system software algorthim. The final interpretation and the susceptibility results were reported by the instrument automatically.

The Statistical Package for the Social Sciences (SPSS) version 22.0, and R environment ver.3.2.2 were used for analysis of the data. Chi-square/ Fisher Exact test was used to find the significance of study parameters on categorical scale between two or more groups in non-parametric setting for qualitative data analysis. p-value less than 0.05 was considered significant.

RESULTS

A total of 36 patients were enrolled in the study. 22(61.1%) were males and 14(38.9%) were females. The mean age was significantly more for males (40.00±14.39 years) compared to females (26.15±8.65 years), p=0.003. The age-wise distribution of the patients is as shown in Figure 1. Maximum number of patients belonged to upper-lower class (15, 41.7%). All the patients had prolonged duration of symptoms. Mean duration of symptoms was 22.44±20.19 months. Maximum number of patients had symptoms for 13 to 24 months (15, 41.67%). 34(94.4%) patients adhered to previously prescribed anti-tubercular treatment. The remaining 2(5.6%) did not adhere to prescribed regimen. Adherence was defined as intake of prescribed regimen until the diagnosis of MDR or duration as prescribed by the treating physician. The mean duration of treatment taken among those who adhered to their regimen was 10.79±5.70 months. Of the 2 patients who were not adherent to treatment, one had taken irregularly for 10 months and the other had stopped treatment at 3 months for 2 courses due to adverse events.

The prescribed regimen was appropriate for 27(75%) patients and inappropriate in the remaining 9(25%).

Appropriate regimen is defined as the Revised National Tuberculosis Control Programme (RNTCP) prescribed 4-drug regimen (Rifampicin, Isoniazid, Ethambutol and Pyrazinamide- RHEZ) for new cases and the now outdated 5-drug regimen (Streptomycin with the other 4 drugs-SRHEZ) for retreatment cases. Use of any of the second line drugs or first line drug in inadequate dose or number of drugs was considered inappropriate.

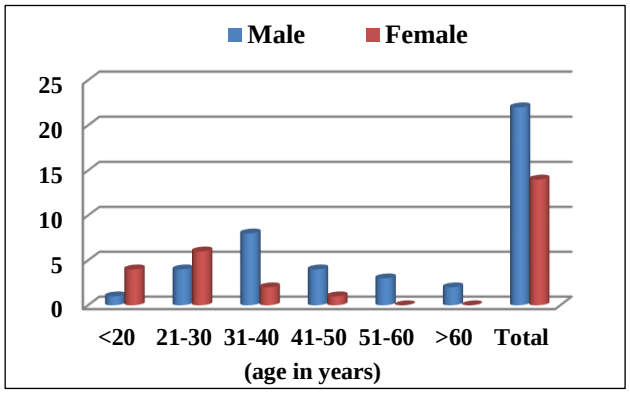

Figure 1: Graph showing age and sex-wise distribution of patients.

Among 26 patients (72.2%) had received multiple courses of ATT. Of these patients, pre-XDR was seen in 13(50%), XDR in 3(11.5%) and MDR in 10(38.5%).

The number of patients who had taken specific antitubercular drugs are shown in table 1. Most of the patients who had been prescribed rifampicin, isoniazid, ethambutol and streptomycin had an appropriate regimen. Fluoroquinolone was the most common inappropriately prescribed drug. 6 of the 9 patients who had received a fluoroquinolone i.e. 66.7% had received it inappropriately. This was found to be statistically significant (p=0.003)

Table 1: Specific anti-tubercular drug received in relation to regimen.

History of anti-tubercular therapy	Regimen		p value
	Appropriate	Inappropriate	
Rifampicin (n=36)	27(75%)	9(25%)	1.000
Isoniazid (n=36)	27(75%)	9(25%)	1.000
Ethambutol (n=36)	27(75%)	9(25%)	1.000
Streptomycin (n=26)	19(73.1%)	7(26.9%)	1.000
Ofloxacin (n=9)	3(33.3%)	6(66.7%)	0.003*

The sensitivity pattern of the first- and second-line drugs in relation to prior intake of the drugs except rifampicin and isoniazid are as shown in table 2. The correlation between resistance to a drug and prior intake of the same drug was not found to be statistically significant for any drug. The p-values are as shown in the tables. Overall, 12(33.3%) patients were found to be MDR, 20(55.6%) were Pre-XDR and the remaining 4(11.1%) were XDR. The correlation of the final diagnosis of MDR, Pre-XDR and XDR with the type of regimen (appropriate/inappropriate) or adherence to the prescribed regimen are shown in tables 3 and 4.

Table 2: Drug sensitivity pattern in relation to history of Anti-Tubercular Treatment (ATT)-Rifampicin and Isoniazid not included.

Drug		Taken	Not taken	Total	p-value
	Resistant	12(33.3%)	0(0.0%)	12(33.3%)	
Ethambutol	Sensitive	24(66.7%)	0(0.0%)	24(66.7%)	1.000
	Total	36	0	36	
	Resistant	14(53.8%)	6(60.0%)	20(55.6%)	
Streptomycin	Sensitive	12(46.2%)	4(40.0%)	16(44.4%)	0.739
	Total	26	10	36	
	Resistant	8(88.9%)	16(59.3%)	24(66.7%)	
Ofloxacin	Sensitive	1(11.1%)	11(40.7%)	12(33.3%)	0.219
	Total	9	27	36	
	Resistant	0(0.0%)	3(8.3%)	3(8.3%)	
Amikacin	Sensitive	0(0.0%)	33(91.7%)	33(91.7%)	1.000
	Total	0	36	36	
	Resistant	0(0.0%)	3(8.3%)	3(8.3%)	
Kanamycin	Sensitive	0(0.0%)	33(91.7%)	33(91.7%)	1.000
	Total	0	36	36	
	Resistant	0(0.0%)	1(2.8%)	1(2.8%)	
Capreomycin	Sensitive	0(0.0%)	35(97.2%)	35(97.2%)	1.000
	Total	0	36	36	

The correlation was not found to be statistically significant (p=0.855 for type of regimen and p=1.000 for adherence).

Among the 4 XDR, 3 were resistant to amikacin, 3 to kanamycin and 1 to capreomycin. None of the patients had received second line injectables in the past.

Table 3: Diagnosis with respect to type of regimen.

Diagnosis	Regimen			
	Total	Appropriate	Inappropriate	
MDR	12(33.33%)	10(37.04%)	2(22.22%)	p=0.855
PRE XDR	20(55.56%)	14(51.85%)	6(66.67%)	
XDR	4(11.11%)	3(11.11%)	1(11.11%)	
Total	36(100%)	27(100%)	9(100%)	

Table 4: Diagnosis with respect to adherence to regimen.

Diagnosis	Total	Adherence		
		No	Yes	
MDR	12(33.3%)	1(50%)	11(32.4%)	p=1.000
PRE XDR	20(55.6%)	1(50%)	19(55.9%)	
XDR	4(11.1%)	0(0%)	4(11.8%)	
Total	36(100%)	2(100%)	34(100%)	

DISCUSSION

Although inappropriate treatment and non-adherence are established causes of resistance, in this study most patients (75%) had received appropriate regimen according to that prescribed by RNTCP and majority (94.4%) had adhered to prescribed treatment. This is contradictory to the study by Rifat et al.[6] where they found drug resistance to be 4 times more likely in patients who did not complete treatment. The study by Sharma et al, found non-compliance to be significantly associated with resistance.[7] A study conducted in Pakistan by Ejaz and colleagues also found the same.[8] This deviation in this study may be explained by the fact that author had enrolled only those patients who had complete record of prior treatment. Such patients with proper records were more likely to have adhered to treatment as well.

In study 72.2% patients had received multiple courses of ATT. Of these patients the most common diagnosis was Pre-XDR (50%). A study by Songhua et al, found that multiple courses of ATT was a significant factor associated with resistance.[9] Many other studies confirm the same.[7,8] The most inadvertently prescribed ATT was found to be Fluoroquinolone (FQ). FQ resistance in Mycobacterium tuberculosis is on the rise. Among the 9 patients who had received FQ, 8(88.9%) were resistant to it. But even in those who had not received it, resistance was seen in 16(59.3%). Thus, correlation of prior use of FQ (specifically for TB) with resistance was not found to be statistically significant (p=0.219). The reason may be that FQ is prescribed for myriad bacterial infections other than TB and cross resistance among the FQ is quite common.[10] FQ is also most commonly used as add on or substitute drug under non programmatic conditions for TB whenever resistance to any drug is proven or suspected or when a person does not tolerate any first line drug.[11] Meta-analyses have demonstrated that exposure to FQ for other reasons before TB diagnosis leads to resistance thereafter in such patients.[12,13] Many other studies from different regions have also demonstrated weak relationship between prior FQ use and FQ resistance.[14-16]

The correlation between resistance to a drug (other than rifampicin and isoniazid) and prior intake of the same drug was not found to be statistically significant for any drug. According to a study by Hamusse et al, individuals with previous history of TB treatment were eight times more likely to develop resistance to any first-line anti-TB drugs compared to those with no history of previous TB treatment.[17] In this study 33.3% of those who had taken Ethambutol were resistant to it. This is exactly similar to the study by Kochi et al, where they found it to be 33.3%. The resistance to streptomycin was more or less similar among those who had and had not taken streptomycin in their study as well authors.[18] None of patients had received second line injectables. But resistance was noted in 4(11.1%) which were also XDR cases. A study from South Korea linked the total duration of Second Line Drug (SLD) intake and lack of measures to ensure adherence and proper monitoring of treatment to the development of XDR.[19] A 3-year prospective study from Russia found that risk of developing resistance to capreomycin and of XDR was higher among MDR patients being treated with regimens containing less than 3 effective drugs compared to those being treated with regimens with more than 3 effective drugs.[20] This does

not apply to this study as none of patients had received any SLD other than FQ.

In this study, 55.6% cases were Pre-XDR and 11.1% were XDR. According to the Global TB report 2018, 8.5% of MDR-TB cases were XDR.[2] Findings correspond to those of Singhal et al, who reported 49.4% pre-XDR and 11.4% XDR in their study.[5] But it is much higher than the 16-32% prevalence of pre-XDR among MDR reported from other parts of Asia.[21,22] The study by Mannan et al, found only 3.4% XDR among MDR.[21] A study from India linked the development of XDR to prior use of SLD and positive family history of TB.[23] Several other factors leading to XDR reported by other studies include younger age, male gender and contact with known MDR for primary cases of XDR and defaults and failures in the past for acquired XDR.[24,25]

Many of the factors mentioned does not hold true for patients because most had received appropriate therapy, most had adhered to treatment, none had contact with DR-TB cases and none had received SLD (except FQ). Hence, primary or intrinsic drug resistance detected after treatment failure is a major probability. This observation needs further exploration.

CONCLUSION

There is higher incidence of DR-TB among retreatment cases. Intake of a drug in the past does not necessarily entail resistance to the same. In fact, the mechanism of evolution of resistance to a drug is complicated and governed by many interplaying factors, most important of which is suboptimal therapy where resistant mutants get selection advantage. Intrinsic (primary) drug resistance is a possibility but that aspect was not analyzed in this study. Hence, first- and second-line DST should be done at diagnosis so that an effective and personalized regimen can be given at the outset. This will prevent treatment failures associated with sticking to a predesigned regimen and improve treatment outcomes and hence reduce burden of DR-TB.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. WHO. Consolidated guidelines on drug-resistant tuberculosis treatment. Geneva: World Health organization;2019. Licence:CCBY-NC-SA3.OIGO. Available at: https://apps.who.int/iris/bitstream/handle/10665/311389/9789241550529-eng.pdf?ua=1. Accessed 30 September 2019.
2. Global tuberculosis report 2018. Geneva: World Health Organization; 2018 WHO/CDS/TB/2018.20. Available at: http://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf. Accessed 2 October 2019.
3. Adwani S, Desai U, Joshi JM. Prevalence of pre extensively drug resistant tuberculosis (Pre XDR TB) and extensively drug resistant tuberculosis (XDR TB) among pulmonary multidrug resistant tuberculosis (MDR TB) at a tertiary care center in Mumbai. JKIMSU. 2016;5:139.
4. Olusoji D, Eltayeb O, Olanrewaju O, Olapade GD. Pre-extensive Drug resistance tuberculosis (Pre-XDR-TB) among MDR-TB Patients in Nigeria. Global Advan Res J Microbiol. 2013;2(2):022-5.
5. Singhal P, Dixit P, Singh P, Jaiswal I, Singh M, Jain A. A Study on Pre-XDR and XDR Tuberculosis and Their Prevalent Genotypes in Clinical Isolates of Mycobacterium Tuberculosis in North India. Ind J Med Res. 2016;145:341-7.
6. Rifat M, Milton AH, Hall J, Oldmeadow C, Islam MA, Husain A, et al. Development of multidrug resistant tuberculosis in Bangladesh: a case-control study on risk factors. PLoS One. 2014;9:e105214.
7. Sharma SK, Turaga KK, Balamurugan A, Saha PK, Pandey RM, Jain NK, et al. Clinical and genetic risk factors for the development of multi-drug resistant tuberculosis in non-HIV infected patients at a tertiary care center in India: a case-control study. Infect Genet Evol. 2003 Sep;3(3):183-8.
8. Ejaz M, Siddiqui AR, Rafiq Y, Malik F, Channa A, et al. Prevalence of multi-drug resistant tuberculosis in Karachi, Pakistan: identification of at-risk groups. Trans R Soc Trop Med Hyg. 2010 Aug; 104(8):511-7.
9. Songhua C, Pengcheng H, Xiaomeng W, et al. Risk factors for multidrug resistance among previously treated patients with tuberculosis in eastern China: a case–control study. Int J Infect Dis. 2013;17:e1116-20.
10. Sirgel FA, Warren RM, Streicher EM, Victor TC, van Helden PD, Bottger EC. gyrA Mutations and Phenotypic Susceptibility levels to Ofloxacin and Moxifloxacin in Clinical Isolates of Mycobacterium tuberculosis. J Antimicro Chemo. 2012;67:1088-93.
11. Murray H, Tay EL, Dinh S, Matthews H, Street A, Denholm JT. The Use of Fluoroquinolones for Tuberculosis in Victoria between 2011 and 2016. Tubercul Res Treat. 2018.
12. van der Heijden YF, Maruri F, Blackman A, et al. Fluoroquinolone susceptibility in Mycobacterium tuberculosis after pre-diagnosis exposure to older- versus newer-generation fluoroquinolones. Int J Antimicro Agents. 2013;42:232-7.
13. Migliori GB, Langendam MW, D'ambrosio L, Centis R, Blasi F, Huitric E, et al. Protecting the tuberculosis drug pipeline: stating the case for the rational use of fluoroquinolones. Eur Respir J. 2012;40:812-22.
14. Shakoor S, Tahseen S, Jabeen K, et al. Fluoroquinolone consumption and -resistance trends in Mycobacterium tuberculosis and other respiratory pathogens: ecological antibiotic pressure and

consequences in Pakistan, 2009-2015. Int J Mycobacteriol 2016;5:412-6.
15. Che Y, Song Q, Yang T, et al. Fluoroquinolone resistance in multidrug-resistant Mycobacterium tuberculosis independent of fluoroquinolone use. Eur Respir J 2017;50:1701633.
16. Devasia RA, Blackman A, Gebretsadik T, Griffin M, Shintani A, May, et al. Fluoroquinoloneresistance in Mycobacterium tuberculosis: the effect of duration and timing of fluoroquinolone exposure. Am J Respir Crit Care Med. 2009;180:365-70.
17. Hamusse SD, Teshome D, Hussen MS, Demissie M, Lindtjørn B. Primary and secondary anti-tuberculosis drug resistance in Hitossa District of Arsi Zone, Oromia Regional State, Central Ethiopia. BMC Pub Health. 2016;16:593.
18. Kochi A, Vareldzis B, Styblo K. Multidrug-resistant tuberculosis and its control. Res Microbiol. 1993 Jan 1;144(2):104-10.
19. Jeon CY, Hwang SH, Min JH, Prevots DR, Goldfeder LC, Lee H, et al. Extensively drug-resistant tuberculosis in South Korea: risk factors and treatment outcomes among patients at a tertiary referral hospital. Clin Infect Dis. 2008 Jan 1;46(1):42-9.
20. Smith SE, Ershova J, Vlasova N, Nikishova E, Tarashova I, Eliseev P, et al. Risk factors for acquisition of drug resistant tuberculosis treatment, Arkhangelsk Oblast, Russia 2005-2010. Emerg Infect Dis. 2015;21:1002-11.
21. Mannan MAU, Mushtaq A, Naqvi S. Prevalence of Pre-XDR-TB, XDR-TB among MDR-TB Patients Registered at PMDT Site Nishtar Hospital Multan Pakistan. Eur Resp J. 2017;50:PA2764.
22. Tasnim T, Tarafder S, Alam FM, Sattar H, Kamal SM. Pre-Extensively Drug Resistant Tuberculosis (Pre-XDR-TB) among Pulmonary Multidrug Resistant Tuberculosis (MDR-TB) Patients in Bangladesh. J Tubercul Res. 2018;6:199-206.
23. Porwal C, Kaushik A, Makkar N, Banavaliker JN, Hanif M, Singla R, et al. Incidence and risk factors for extensively drug-resistant tuberculosis in Delhi region. PLoS One. 2013;8:e55299.
24. Ignatyeva O, Balabanova Y, Nikolayevskyy V, Koshkarova E, Radiulyte B, Davidaviciene E, et al. Resistance profile and risk factors of drug resistant tuberculosis in the Baltic countries. Tuberculosis. 2015;95:581-8.
25. World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. WHO/HTM/TB/2014.11. Geneva: WHO; 2014. Available at: https://www.who.int/tb/publications/pmdt_companionhandbook/en/.

Cite this article as: Kumar R, Garg R, Kshetrimayum S, Jain A. Drug resistance profile among multi-drug resistant tuberculosis patients at diagnosis and correlation with history of anti-tubercular treatment in a tertiary care center. Int J Adv Med 2019;6:1918-23.