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Pattern of resistance to second line anti-tuberculosis drugs and associated risk factors among multidrug Resistant patients of pulmonary tuberculosis from Agra region of Uttar Pradesh, North India

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Abstract

Present study was aimed to determine the status of second-line anti-TB drug resistance and risk factors for extensively drug-resistance (XDR)-tuberculosis among multi-drug resistant (MDR)-tuberculosis patients from Agra region of Uttar Pradesh, North India. A total of 279 MDR-*Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients of Agra region of Uttar Pradesh were analyzed for the resistance to second-line anti-TB drugs (SLDs). The demographic, behavioral, clinical and social information was compared with the results of drug susceptibility testing and analyzed statistically. Out of 279 MDR-isolates, 195 (69.89%) isolates were found resistant to at least one of the SLDs. The rates of general resistance to each of the drugs were as follows: kanamycin (42.29%), amikacin (17.92%), levofloxacin (40.14%) and ofloxacin (51.97%). Among the MDR-TB isolates, 53.76, 43.01 and 26.88% isolates were resistant to fluoroquinolone, second line injectable drugs and XDR, respectively. Among the demographic/clinical variables, occupation, gender and number of family members were associated with the development of XDR-TB in MDR-TB patients of Agra region of Uttar Pradesh (P value: <0.05). Present study has provided baseline information about the risk factors associated with SLDs resistance and XDR among MDR-TB patients which can be useful to strengthen the implementation of targeted interventions in order to minimize the cases of drug resistance tuberculosis in Uttar Pradesh, North India.

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Introduction

Emergence of drug resistance among *Mycobacterium tuberculosis* strains is a dangerous alarm for the effective management of tuberculosis (TB) worldwide. The

multidrug-resistant (MDR: resistance to both Isoniazid (INH) and rifampicin (RIF) with or without for other drugs) and extensively drug-resistant (XDR: resistance to at least INH and RIF with additional resistance to any fluoroquinolone and at least one of three injectable anti-TB drugs) form of TB presents tremendous challenges to the national TB elimination programs worldwide. In 2019, an estimated 3.3% of new TB cases and 18% of previously treated cases had multi drug / rifampicin resistant TB (MDR/RIF resistant -TB) worldwide [1]. As per the WHO estimates, a total of 4, 65,000 cases of MDR/RR-TB have occurred in 2019 and 182,000 people died of MDR/RR-TB globally [1]. India has the highest burden of MDR-TB cases followed by China and accountable for about 27% of global MDR/RR-TB cases. The report of "First National Anti-Tuberculosis Drug Resistance Survey" revealed that MDR-TB is 6.19% among all TB patients with 2.84% among naive and 11.60% among previously treated TB patients in India. Among MDR-TB patients in India, additional resistance to any fluoroquinolones was found as 21.82% and 3.58% to any second-line injectable drugs. The burden of TB and MDR-TB distributed unequally between different parts of the country and between the states in India [2]. The Uttar Pradesh (UP) has highest burden of TB and drug resistant TB among the 28 states and 8 Union territories in India. Despite the highest burden of TB and MDR-TB, information on status and determinants associated with the development of drug resistant TB i.e. XDR-TB is limited in Uttar Pradesh [3]. The systematic knowledge about the local variations in diseases status and risk factors responsible for high burden of disease is required in order to provide evidence for planning of the targeted programmatic interventions to reduce the burden of drug resistant-TB in Uttar Pradesh, North India. Present study was aimed to identify the status of second line drug resistance and risk factors for XDR-TB among MDR-TB patients in Agra region of Uttar Pradesh, North India.

Material and Methods

Present study was carried out at Department of Microbiology and Molecular Biology, ICMR-National JALMA Institute for Leprosy and other Mycobacterial Diseases, Agra, India, during the period of January 2017 to January 2020. The written informed consent was obtained from all the study participants, before collection of clinical samples and personalized information.

Mycobacterium tuberculosis isolates

During the period of 2017-2020, a total of 279 MDR isolates were recovered from sputum samples of suspected MDR-TB patients of Uttar Pradesh, North India and included in this study. After written informed consent, the demographic and clinical characteristics of all the study participants were recorded (Table 1). The isolates were recovered from the sputum samples on Lowenstein-Jensen (LJ) medium as per the method described by Raizada *et al.*, [4]. The species level identification of the isolates was performed using biochemical tests as per the method of Vestal [5]. The drug susceptibility patterns of *M. tuberculosis* isolates was studied against first line (rifampicin, isoniazid, ethambutol and streptomycin) and second line (kanamycin, ofloxacin, amikacin, and levofloxacin) anti-TB drugs using minimum inhibitory concentration method on LJ-medium as per the guideline of national elimination tuberculosis control program, India. The procedure of isolation of mycobacteria and drug susceptibility testing were conducted in Biosafety level-3 (BSL-3) laboratory, Department of Microbiology and Molecular Biology, ICMR-NJIL&OMD, Agra.

Identification of risk factors and data analysis

The demographic information, clinical/epidemiological data were compared with the microbiological results of individual study participants for the identification of risk factors for XDR-TB. The criterion for significance was set at p value <0.05.

Results and Discussion

In present study, status of second-line anti-TB drug resistance and risk factors for XDR-TB among MDR-TB patients of Agra region of Uttar Pradesh was studied. Of the 279 MDR *M. tuberculosis* isolates, 84 (30.11%) and 195 (69.89%) isolates were found pan-susceptible and resistant to at least one of the second line anti-TB drug, respectively (Fig. 1). The rate of any resistance to kanamycin, amikacin, levofloxacin and ofloxacin was 42.29, 17.92, 40.14 and 51.97%, respectively (Fig. 1) previous studies from various parts of the India have reported variable prevalence of second line anti-TB drugs (3 to 35%) among the MDR-TB patients [6-9]. In present study, the higher proportion of MDR-TB isolates was found resistant to fluoroquinolones (53.76%) as compare to second line injectable drugs (43.01%). Similar to the present study, Singh and Jain [10] also reported higher prevalence of fluoroquinolones as compare to second line injectable drugs among MDR/Rifampicin resistant-TB isolates in Uttar Pradesh. Mamatha and Shanthi [11] reported 32% of FQ resistance among MDR-TB isolates in South India. The variation in

the proportion of second line drug resistance among the studies may be due to the geographical differences, variation in the methodology and period of studies. Previously, Agrawal *et al.*, [6] analyzed the incidence of fluoroquinolone (FQ) resistant *M. tuberculosis* and reported increasing trends as 3% in 1996 to 35% in 2004 in Mumbai, North India. The higher prevalence of fluoroquinolone as compared to second line injectable drugs may be attributed to easy access and indiscriminate use of fluoroquinolones for the treatment of other common non-TB infections in India [12].

In present study, the proportion of XDR-TB among the MDR-TB isolates was 26.88%. Previously, Balaji *et al.*, [13] also outlined high incidence of XDR-TB (23%) among pulmonary TB patients in India. However, Sharma *et al.*, [8] found 2.4% of MDR-TB cases as XDR- from Delhi, North India. In another study from Delhi region Khanna *et al.*, [14] reported 5.76% XDR-TB among MDR-TB patients. Porwal *et al.*, [9] reported 3.7% *M. tuberculosis* as XDR among MDR-TB patients in Delhi Region of North India. Since, the proportion of drug resistant TB depends on study design, sampling frame and study population. The high prevalence of XDR-TB in present study may be due to presence of large pool of samples taken from patients visited drug resistant -TB center, S.N. Medical College, Agra for anti-TB treatment and patients with previous history of TB treatment which is well known factor for increased risk of drug resistant -TB development [15, 16].

The predictors for XDR -TB in the study population were further studied. In present study, occupation (P value: <0.002), gender (P value: <0.001) and number of family members (P value: <0.010) were found significant predictors for XDR-TB among MDR-TB patients (Table 2). The prevalence of XDR-TB was found higher among housewives (48.94%) followed by unemployed (31.11%) farmers (23.81%) and laborers (20.0%). Similar to the present study, Thakurathi *et al.*, [17] also reported high risk of MDR/XDR-TB in housewives in Nepal. In present study, prevalence of XDR-TB was found higher in female (38.0%) as compare to male (20.67%) and the difference was statically significant (P value: <0.001). The result of this study was in accordance of previous study reported female gender as a risk factor of XDR-TB [18]. The higher prevalence of XDR-TB in female may be due to the reason that women find great barriers for healthcare and suffer from stigma and social consequences of drug resistant TB as compared to men. In present study, higher prevalence of XDR-TB was found in patients of middle age (26-45 years), resident of urban areas, with the history of cough,

smoking and diabetes illness, previous history of TB treatment, but the difference was statistical insignificant (Table 3). Similar to the present study, previous studies also have not found any significant association between DR-TB and age [19, 20]. Similar to the present study, previous studies have reported treatment history as an important predictor for the development of XDR-TB [21]. In present study, no significant association was found between the alcohol intake and XDR-TB. However, previous studies showed that alcohol consumption leads to poor adherence to treatment, impaired immune response and leading to increased risk of acquiring XDR-TB [22, 23]. The variation in the identified risk factors among the studies may be due to the geographical differences, variation in the sample size and period of studies. The present study has some limitations. The major limitation of study is being conducted in hospital setting and may not be representative of the study groups in general population at the large.

Table 1: Socio-demographic and clinical characteristics of MDR-TB patients

Variable	Response	Number of patients (n=279)	
		Frequenc y	Percent (%)
Occupation	Farmers	21	7.53
	House wife	47	16.85
	Labourers	45	16.13
	Unemploye d	45	16.13
	Others	121	43.36
Gender	Male	179	64.16
	Female	100	35.84
Age	Up to 25 years	114	40.86
	26-45 years	109	39.07
	Above 45	56	20.07
Living conditions	Rural	99	35.48
	Urban	180	64.52
Number of family members	1 to 5	123	44.09
	6 to 10	121	43.37
	Above 10	35	12.54
Economic status (Indian rupees per person per month)	Group 1 <1000	25	8.96
	Group 2 1001-2000	19	6.81
	Group 3 >2000	138	49.46
	No	97	34.77

	information provided		
Literacy	Illiterate	105	37.63
	Below high school	96	34.41
	High school & above	78	27.96
Household contacts	Yes	60	21.51
	No	219	78.49
Patients with cough	Yes	222	79.57
	No	57	20.43
Weight loss	Yes	235	84.23
	No	44	15.77
Status of HIV infection	Yes	3	1.08
	No	242	86.74
	No information available	34	12.19
Status of Diabetes illness	Yes	8	2.87
	No	261	93.55
	No information available	10	3.58
Previous history of TB treatment	Yes	176	63.08
	No	103	36.92
Smoking History	Yes	67	24.01
	No	212	75.99
Alcohol Uptake History	Yes	63	22.58
	No	216	77.42
Hospitalization History	Yes	48	17.20
	No	231	82.80

Table 2: Risk factors associated with extensive drug resistance (XDR) among the study population in North India.

Variables	Response	Total (n=279)	XDR (n=75)	P-value
			No. isolates (%)	
Occupation	Farmers	21	5 (23.81)	0.002
	House wife	47	23 (48.94)	
	Laborers	45	9 (20.00)	
	Unemployed	45	14 (31.11)	
	Others	121	24 (19.83)	

Gender	Male	179	37 (20.67)	0.001
	Female	100	38 (38.00)	
Age	Up to 25 years	114	31 (27.19)	0.088
	26-45 years	109	35 (32.11)	
	Above 45	56	9 (16.07)	
Living conditions	Rural	99	20 (20.20)	0.061
	Urban	180	55 (30.56)	
Number of family members	1 to 5	123	43 (34.96)	0.010
	6 to 10	121	28 (23.14)	
	Above 10	35	4 (11.43)	
Economic status (Indian rupees per person per month)	Group 1 <1000	25	7 (28.00)	0.164*
	Group 2 1001-2000	19	1 (5.26)	
	Group 3 >2000	138	28 (20.29)	
	No information provided	97	39 (40.21)	
Literacy	Illiterate	105	25 (23.81)	0.071
	Below high school	96	21 (21.88)	
	High school & above	78	29 (37.18)	
Household TB contacts	Yes	60	14 (23.33)	0.484
	No	219	61 (27.85)	
Patients with cough	Yes	222	60 (27.03)	0.913
	No	57	15 (26.32)	
Weight loss	Yes	235	65 (27.66)	0.498
	No	44	10 (22.73)	
Status of HIV infection	Yes	3	0 (0.00)	-
	No	242	65 (26.86)	
	No information available	34	10 (29.41)	
Status of Diabetes illness	Yes	8	3 (37.50)	0.469*
	No	261	68 (26.05)	
	No information available	10	4 (40.00)	
Previous	Yes	176	52 (29.55)	0.18

history of TB treatment	No	103	23 (22.33)	9
Smoking History	Yes	67	19 (28.36)	0.75
	No	212	56 (26.42)	4
Alcohol	Yes	63	16 (25.40)	0.76

Uptake History	No	216	59 (27.31)	2
Hospitalization History	Yes	48	16 (33.33)	0.26
	No	231	59 (25.54)	7

*Based on the patients with information available only.

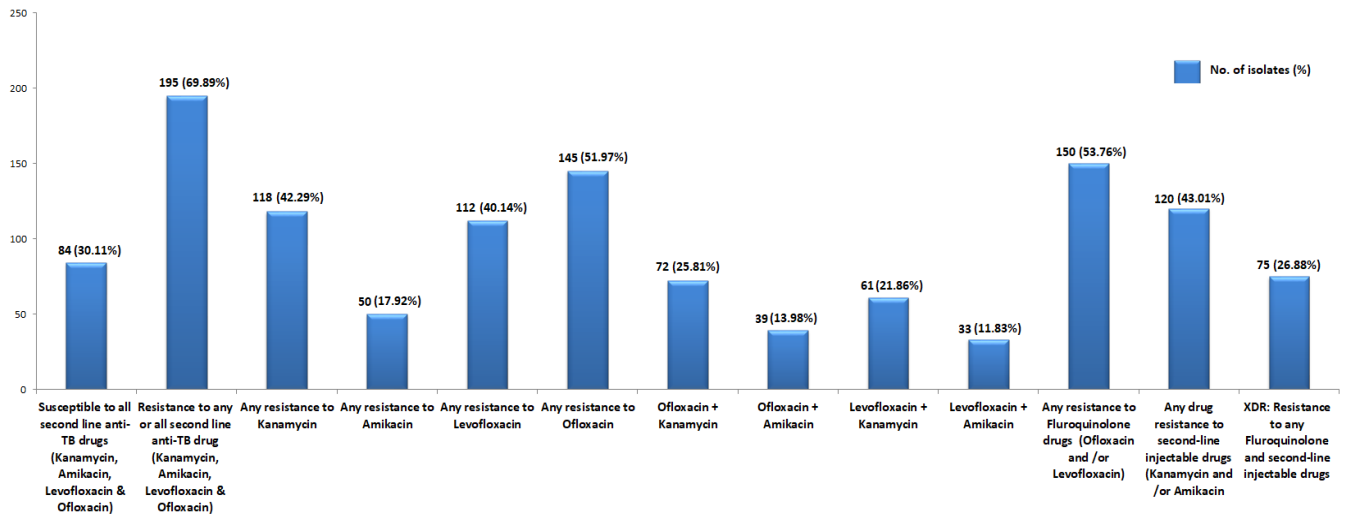


Fig 1: Second line anti-tuberculosis drug resistance patterns of MDR-isolates of M. tuberculosis (n=279)

Table 3: Second line anti-tuberculosis drug resistance patterns of MDR-isolates of M. tuberculosis (n=279)

Pattern of drug resistance	Total number of isolates	Percent (%)
Susceptible to all second line anti-TB drugs (Kanamycin, Amikacin, Levofloxacin & Ofloxacin)	84	30.11
Resistance to any or all second line anti-TB drug (Kanamycin, Amikacin, Levofloxacin & Ofloxacin)	195	69.89
Any resistance to Kanamycin	118	42.29
Any resistance to Amikacin	50	17.92
Any resistance to Levofloxacin	112	40.14
Any resistance to Ofloxacin	145	51.97
Ofloxacin + Kanamycin	72	25.81
Ofloxacin + Amikacin	39	13.98
Levofloxacin + Kanamycin	61	21.86
Levofloxacin + Amikacin	33	11.83
Any resistance to Fluroquinolone drugs (Ofloxacin and /or Levofloxacin)	150	53.76
Any drug resistance to second-	120	43.01

line injectable drugs (Kanamycin and /or Amikacin)		
XDR: Resistance to any Fluroquinolone and second-line injectable drugs	75	26.88

Conclusions

Present study reported high rates second line drug resistance among MDR-TB patients in Agra region of Uttar Pradesh, North India. The occupation (i.e. housewives, unemployed, farmers and laborers) and female gender seems to be important risk factors for XDR-TB among MDR-TB patients in study setting. Study highlights the need of improved strategies for the screening and effective TB control among MDR-TB patients to reduce the burden of second line drug resistant TB in Uttar Pradesh North India.

Conflict of Interest

Nil

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