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Drug susceptibility pattern of Mycobacterium tuberculosis in patients with pulmonary tuberculosis

KEYWORDS: Tuberculosis, Drug resistance.

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ABSTRACT

BACKGROUND: *The literature regarding drug resistant tuberculosis (DRTB) is scanty in most of the regions of the country. Hence the present study was undertaken to understand the drug susceptibility pattern of Mycobacterium tuberculosis (MTB) in patients with pulmonary tuberculosis (PTB).*

MATERIALS AND METHODS: *Early morning satisfactory sputum sample was collected and subjected to sputum smear microscopy after Ziel-Neelsen (ZN) staining as per Revised National Tuberculosis Control Programme (RNTCP) guidelines. All the sputum samples were delivered by courier in a cold chain to Dr. Iravatham's Clinical Laboratory, Hyderabad and were processed using the N-acetyl-L-cysteine - sodium citrate – sodium hydroxide method. Sediment was subjected to ZN staining, inoculated on Lowenstein-Jensen media for 8 weeks and Line Probe Assay (LPA) (according to the manufacturer's instructions (Genotype MTBDRplus, Hain Life-Science, Nehren, Germany).*

RESULTS: *Out of 67 cases enrolled in our study, resistance to Isoniazid (H) is seen in 4 cases (5.97%), resistance to Rifampicin (R) in 18 cases (26.86%), resistance to both H and R in 22 cases (32.83%). The sensitivity to both H and R is seen in 20 cases (29.85%).*

CONCLUSION: *Rate of drug resistance PTB is high in patients been on irregular treatment and patients previously treated.*

INTRODUCTION:

Tuberculosis (TB) is an infectious disease caused by MTB. [1] No organ is immune to TB, PTB being the most common, accounting for more than 85% of all the TB cases. [1] The source of infection is a person with sputum smear positive (SSP) PTB. [1] Transmission occurs by the airborne spread of infectious droplets and droplet nuclei containing the tubercle bacilli. [1]

In 2007, an estimated 13.7 million cases were active globally. [2] In 2013, an estimated 9 million new cases occurred globally [3] and there were between 1.3 and 1.5 million associated deaths. [4] In 2012, out of the estimated global annual incidence of 8.6 million TB cases, 2.3 million were estimated to have occurred in India and mortality rate was 22 per 100,000 persons. [5] There is 42% reduction in TB mortality rate by 2012 as compared to 1990 level. Similarly there is 51% reduction in TB prevalence rate by 2012 as compared to 1990 level. [5]

The emergence of DRTB has become a significant public health problem worldwide and an obstacle to effective TB control, particularly multi drug resistant (MDR) TB and extensively drug resistant (XDR) TB. Globally, 5% of TB cases were estimated to have had MDR TB in 2013 (3.5% of new and 20.5% of previously treated TB cases). Drug resistance surveillance data show that an estimated 480,000 people developed MDR-TB in 2013 and 210,000 people died. XDR-TB has been reported by 100 countries in 2013. On average, an estimated 9% of people with MDR-TB have XDR-TB. [6] If all notified TB patients (6.1 million, new and previously treated) had been tested for drug resistance in 2013, an estimated 300,000 cases of MDR-TB would have been detected. In 2013, 136,000 of the estimated 300,000 MDR-TB patients who could have been detected were diagnosed and notified. This represents a tripling in MDR-TB detection compared with 2009. [6] In India during 2013, the percentage of MDR-TB cases among new and retreated cases was 2.2% and 15% respectively, MDR-TB cases among notified new and retreated PTB cases was 20,000 and 41,000 respectively. [7]

Previous treatment for TB is the strongest determinant for DRTB [8,9,10,], but even naive patients are also at the risk of developing DRTB because of genetic mutations or transmission of resistant bacilli. [11,12,]

MTB undergoes constant and spontaneous but slow mutations resulting in resistant mutant bacilli. [12,13,] This phenomenon is genetically determined and is variable for different anti-tubercular drugs. Inside a lung cavity of diameter 2.5 cm the number of MTB are found in the order of 100 million, i.e 108. [14] As a rule of thumb, the average frequency of resistant mutant bacilli is ~1 in 106 to Isoniazid (H) and ~1 in 108 to Rifampicin (R). Thus occurrence of bacilli resistant to both H+R is ~1 in 1014 bacilli. [14] This illustrates that DRTB is a man-made phenomenon. A high bacterial load and several cycles of inadequate treatment (poor treatment, poor drugs and poor adherence) are therefore needed for significant numbers of drug resistance bacilli to emerge (acquired drug resistance). These resistant strains of bacilli can also be transmitted to individuals who previously never had TB and can present with DRTB (primary drug resistance). Based on drug susceptibility testing (DST) of clinical isolates confirmed to be MTB, DRTB can be classified as:

1) Mono drug resistant - resistance to one first-line anti-TB drug only.
2) Poly drug resistant - resistance to more than one first-line anti-TB drug (other than H+R).

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3) MDR - resistance to at least both I and R.
4) XDR - resistance to any fluoroquinolone and to at least one of three second-line injectable

drugs (Capreomycin, Kanamycin and Amikacin), in addition to MDR. [15]

The literature regarding DRTB is scanty in most of the regions of the country. Hence the present study was undertaken to understand the drug susceptibility pattern of MTB in patients with PTB.

MATERIALS AND METHODS:
This study is an institutional based, single center, prospective study.

INCLUSION CRITERIA:
1) Patients consenting for the study.
2) Sputum smear positive (SSP) PTB cases.
3) Age ≥18 years.

EXCLUSION CRITERIA:
1) Patients not consenting for the study.
2) Extra pulmonary TB.

Attaining Institutional Ethical Committee clearance, SSP-PTB cases diagnosed as per RNTCP guidelines attending Department Of Pulmonary Medicine, Navodaya Medical College Hospital and Research Centre - Raichur, including both in-patients and out-patients from 1st October 2011 to 30th November 2014, after informed written consent were enrolled in our study.

Early morning satisfactory sputum sample was collected in a wide mouthed capped sterile container and subjected to sputum smear microscopy after ZN staining as per RNTCP guidelines. All the sputum samples were delivered by courier in a cold chain to Dr. Iravatham's Clinical Laboratory, Hyderabad and were processed using the N-acetyl-L-cysteine - sodium citrate – sodium hydroxide (NALC-NaOH) method [16] and sediment was subjected to ZN staining, inoculated on LJ media for 8 weeks and LPA according to the manufacturer's instructions (Genotype MTBDRplus, Hain Life-Science, Nehren, Germany).

The LPA was performed according to the manufacturer's protocol. [17] LPA is based on DNA strip technology and has three steps: 1) DNA extraction, 2) Multiplex PCR amplification, and 3) Reverse hybridization. All three steps were performed as per the WHO recommendations. [18]

RESULTS:
The total numbers of patients enrolled in our study were 67.

AGE DISRRIBUTION:
The patients between the age group of 11-20 years were 3 (4.47%), 21-30 years were 19 (28.35%), 31-40 years were 11 (16.41%), 41-50 years were 17 (25.37%), 51-60 years were 13 (19.40%), 61-70 years were 2 (2.98%), 71-80 years were 2 (2.98%).

GENDER DISTRIBUTION:
Males constituted 59.71% (40/67) and females 40.29% (27/67).

SPUTUM AFB - ZN STAINING (DIRECT):
Among the 67 cases enrolled in the study, sputum for AFB by ZN staining technique (direct) was 3+ in 17 cases, 2+ in 29 cases, 1+ in 13 cases, scanty 8 cases.

HABITS:
18 (26.86%) cases had history of substance abuse. History of smoking is present in 11 cases, alcoholic beverage consumption in 4 cases and tobacco chewing in 1 case. The combination of smoking and alcoholic beverage consumption in 1 case while smoking and tobacco chewing in 1 case.

COMORBIDITY:
12 (17.91%) cases had comorbidities. Diabetes mellitus (DM) type 2 in 7 cases, systemic hypertension (HTN) type 2 in 2 cases, chronic obstructive disease (COPD) in 2 cases and anaemia in 1 case.

HISTORY OF PREVIOUS TREATMENT:
TABLE-1

HISTORY OF PREVIOUS TREATMENT							
NO	YES						
	DAILY	RNTCP					
		CAT 1			CAT 2		
		R	D	F	R	D	F
26	C 4	5	3	2	4	17	6
		10			27		
	4	37					
	41						

[C- Cure, R-Relapse, D-Lost for follow up, F-Failure]

There was no history of previous treatment for TB in 26 cases (38.80%). History of previous treatment for TB was present in 41 cases (61.19%). Among which 4 cases (4/41, 9.75%) were treated with daily regimen, all 4 cases were declared as "cured" at the end of the treatment. 37 cases (37/41, 90.24%) were treated under RNTCP, of which 10 cases (10/41, 24.39%) were treated with category-1 under RNTCP Directly Observed Treatment, Short course (DOTS), 27 cases (27/41, 65.85%) were treated with category-2 under RNTCP DOTS. [Refer table -1]

SPUTUM AFB - ZN STAINING (CONCENTRATION METHOD):
All the sputum samples subjected to ZN staining after subjecting it to NALC-NaOH method all the sputum samples were smear positive with grade 3+ in 23 (34.32%) cases, 2+ in 30 cases (44.77%), 1+ in 14 cases (20.89%).

LJ CULTURE:
All the 67 sputum samples were subjected to LJ culture, of which 64 (95.52%) were positive and 3 (4.47%) were negative.

DRUG SENSITIVITY PATTERN:
TABLE-2

DRUG SENSITIVITY PATTERN			
RESISTANCE			
	DRUGS	NO	%
	H	4	5.97
	R	18	26.86
	H+R	22	32.83
SENSITIVE			
	DRUGS	NO	%
	H+R	20	29.85
CULTURE	NEG	3	4.47
TOTAL		67	100

The resistance to H is seen in 4 cases (5.97%), resistance to R in 18 cases (26.86%), resistance to both I and R in 22 cases (32.83%). The sensitivity to both H and R is seen in

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20 cases (29.85%). [Refer table-2]

TREATMENT HISTORY AND DST PATTERN:
TABLE-3

PREVIOUS TREATMENT HISTORY								
NO				YES				
RESISTANCE			SENSITIVE	RESISTANCE			SENSITIVE	CULTURE NEG
H	R	H+R	H+R	H	R	H+R	H+R	
1	4	2	19	3	14	20	1	3
7			19	37			1	3
26				41				
67								

DRTB was observed among 26.92% (7/26) patients with no previous history of ATT, being 14.28% (1/7) for H, 57.14% (4/7) for R and 28.57% (2/7) for H+R. and 98.24% (37/41) patients with previous history of ATT. DRTB among patients previously treated with ATT is 98.24% (37/41), 8.10% (3/37) for H, 37.83% (14/37) for R and 54.05% (20/37) for H+R. [Refer table - 3]

DISCUSSIONS:
The total number of the patients enrolled in our study is 67. The majority of the patients, 19 (28.35%) were in the age group between 21-30 years, the maximum age being 72 years and minimum age being 18 years in our study. The males constituted the majority 59.71% (40/67), whereas females constituted 40.29% (27/67).

Our findings are consistent with other studies like Haji Khan Khoharo et al [19] where 285 cases were enrolled, 176 (61.75%) were male and 109 (38.24%) female. The mean age was 37 ± 19.90 years. Saugat et al [20] where 100 patients were enrolled, the mean age of our cohort was 41.7 years whereas mean age for males and females was 44.14 years and 37.54 years, respectively. T Dam et al [21] enrolled 263 patients between the age group of 20-70 years from 181 (68.82%) males and 82 (31.17%) females.

All the 67 cases enrolled in our study were SSP-PTB as per RNTCP guidelines with grade of 3+ in 17 (25.37%) cases, 2+ in 29 (49.23%) cases, 1+ in 13 (19.40%) cases and scanty in 8 (11.94%) cases but when sputum was subjected to ZN staining after processing it with NALC-NaOH method, SSP with grade 3+ in 23 (34.32%) cases, 2+ in 30 cases (44.77%), 1+ in 14 cases (20.89%) and none as scanty.

Our finding on digestion and decontamination of sputum for AFB is consistent with study conducted by Farina et al [22] where NALC-NaOH method has been shown to be a sensitive and reliable method for microscopy and culture of AFB.

Of 67 cases, 18 (26.86%) cases had history of substance abuse. Tobacco smoking being the commonest, 13 cases (13/67, 19.40%). This shows a strong association between PTB and tobacco smoking which is consistent with various studies conducted to establish the relationship between tobacco smoking and PTB. [23,24,]

Of the 67 cases enrolled, 12 (17.91%) cases had comorbidities, most common being DM -type 2 detected in 7 cases (7/67, 10.44%).

All the 67 sputum samples were subjected to LJ culture of which 3 (4.47%) sputum samples turned out to be culture negative at the end of 8 weeks incubation.

In our study mono drug resistance to H is detected in 4 cases (5.97%). This is comparatively the lowest when compared to H resistance detected in studies conducted at Mysore 20.8% [25], Chandigarh 14.3% [26] and Gujarat 11.7% [27]

In our study mono drug resistance to R is detected in 18 cases (26.86%). Resistance to R is considered as the surrogate marker of MDR TB. [28] Similar findings are reported from Chandigarh 27.6% [26], Mysore 28% [25], New Delhi 33.3% [29] and relatively higher in Haryana 49% [30] and Raichur 100% [31].

The MDR cases detected in our study are 22 (32.83%). Our study is consistent with MDR PTB cases detected in the study conducted at Gujarat (30.2%). [32] The MDR PTB cases detected in studies conducted across India are as follows Tamil Nadu (25%) [33], Mumbai (25.25%) [34], Mysore (25.61%) [25], Chandigarh (27.6%) [26] and Delhi (53.6%) [35]. The highest rate was observed in Dehradun (57.22%) [36], the lowest rates were seen in Sewagram Wardha (9.2–9.6%) [37].

In our study 41 (61.19%) cases had past history of treatment for TB. There is high level of DRTB among patients been on irregular treatment and patients previously treated, being 9.75% (4/41) for H, 34.14% (14/41) for R and 48.78% (20/41) for H+R. Similar finding have been reported by Sethi et al, 46.9% to H, 27.6% to R [26]. A review of various studies from India by Paramasivan et al, regarding drug resistance in TB have also shown high resistance to H (47–87.1%) and R (12.6-80.6%) in previously treated cases. [9]

High prevalence of DR-PTB in our study may be due to the clustering of seriously ill and or referred cases at our tertiary care center.

Limitations of the study:
Sample size is small which do not represent whole community.

Single center study.

Drug susceptibility of MTB for second line ATT was not included in the study due to financial constraints.

CONCLUSONS:
Rate of drug resistance PTB is high in patients been on irregular treatment and patients previously treated. Hence DRTB can be prevented by awareness, early diagnosis, rapid accurate DST, implementation of recommended treatment guidelines, timely monitoring of the patients and making sure therapy is completed.

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