RESISTANCE PATTERNS IN PATIENTS OF DRUG RESISTANT PULMONARY TUBERCULOSIS

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ABSTRACT

Objective: To study the patterns of resistance to anti tuberculosis drugs in patients of drug resistant pulmonary tuberculosis.

Study Design: Cross sectional analytical study.

Place and Duration of Study: Department of Pulmonology, PEMH Rawalpindi from Jul 2016 to Dec 2017.

Methodology: Four hundred and eighty eight (n=488) adult patients with suspected pulmonary tuberculosis based on clinical history or Chest X-ray findings suggestive of pulmonary TB were included in the study after full informed consent and using consecutive sampling. Patients were evaluated with examination of sputum or/and endobronchial washings for AFB smear, MTB GeneXpert/RIF assay and Culture for mycobacterium tuberculosis with drug susceptibility testing for first and second line anti TB drugs. Patients with resistance to any anti TB drug were classified as drug resistant tuberculosis cases and pattern of drug resistance documented.

Results: Overall 74 patients were found to have drug resistant tuberculosis. Treatment naïve patients made up 48.6% of the drug resistant cases while 51.4% of the patients of drug resistant cases were previously treated cases. Among the drug resistant TB patients, 29 Patients (39.2% of the DR TB cases) were found to have MDR TB while 28 patients (37.8%) were found to have mono resistance tuberculosis. Resistance to INH was the commonest being present in 57 (77% of DR TB cases) patients which included 25 patients with mono INH resistance (89.3% of mono drug resistant cases). Fourteen patients (18.9% of DR TB) were found to have rifampicin resistance (RR-TB) on GeneXpert/RIF assay alone. Fluoroquinolone resistance was present in 22 patients (29.7% of the DR TB cases) while resistance to second line injectables was detected in three patients (4.1% of DR TB cases) including two patients diagnosed a XDR TB.

Conclusion: Drug resistant pulmonary tuberculosis was frequent in previously treated as well as new patients of pulmonary tuberculosis. Drug susceptibility testing including molecular methods for detection of resistance to first line and second line drugs are essential for optimum management of these cases.

Keywords: Drug resistant tuberculosis, Drug susceptibility test, Multi drug resistant tuberculosis.

INTRODUCTION

Drug resistant tuberculosis especially Multi drug resistant (MDR) tuberculosis and extensive drug resistant (XDR) TB is a global health problem with increasing numbers of people being affected in developing countries[1]. World health organization (WHO) classifies drug resistant TB cases based on Drug susceptibility testing (DST) on clinical isolates as;[2]

- Mono-resistance: Resistance to one first-line anti-TB drug only.

- Poly-resistance: Resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin.

- Multidrug resistance: Resistance to at least both isoniazid and rifampicin.

- Extensive drug resistance: resistance to any Fluoroquinolone (FQ), and at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance.

- Rifampicin resistance: Resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs.

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In 2016, WHO estimated 10.4 million new TB cases worldwide and 600000 new cases with rifampicin resistance of which 490000 cases were reported as MDR-TB[3]. Pakistan remains among the top 30 high TB burden countries and an estimated 15000 cases were notified as MDR or RR-TB in 2016[3]. Improper administration and failure to ensure completion of anti-tuberculosis therapy (ATT) are the most important causes of drug resistant tuberculosis. Poverty, illiteracy and inadequate TB control programs have contributed to high prevalence of drug resistant tuberculosis in different populations[4].

The critical determinant of treatment success in a tuberculosis patient is drug resistance. Even in cases of mono drug resistant TB like mono Isoniazid (INH) resistant tuberculosis, treatment with first line drugs may be sub-optimal resulting in treatment failure, and contribute to multi drug resistant epidemic[5]. Determination of changes in drug resistance patterns in a community is therefore very important to ensure treatment success. The objective of this study was to determine the pattern of resistance in patients with drug resistant tuberculosis.

METHODOLOGY

This cross sectional analytical study was carried out in the department of Pulmonology, Pak Emirates Military Hospital, Rawalpindi from July 2016 to December 2017. Sample size was calculated as recommended by WHO considering the estimated proportion of drug resistant TB as 11.5 percent[4], margin of error of 0.05, confidence level of 95% and expected response rate of 0.8. Four hundred and eighty eight adult patients suspected of pulmonary TB on the basis of clinical history and/or Chest x-rays suggestive of pulmonary TB, including previously treated patients presenting with signs and symptoms of relapse or re-infection, were included in the study by consecutive non-probability sampling. Patients less than 12 years of age were excluded from the study.

The patient's history and medical documents were used to note the demographic data and drug history. Patients were classified according to their treatment history into two groups. New cases were defined as those who had never received treatment or received ATT for less than 4 weeks. Previously treated cases were those who had received ATT for at least 4 weeks and included patients with relapse, treatment failure and treatment after loss to follow up.

Informed written consent was taken from all patients. Two Sputum specimen for microbiological examination, were collected in the presence of a doctor including one Spot sample and sent for Gene-Xpert/RIF assay, AFB smear and mycobacterial culture with drug susceptibility test (DST). Patients who were unable to produce sputum were offered bronchoscopy with bronchial washings/ Bronchoalveolar lavage and samples were sent for Xpert/RIF assay and culture with DST. Patients who were found to have resistance to any anti TB drug on the basis of Xpert / RIF assay or DST were classified as Drug resistant TB cases and the pattern of drug resistance noted.

The data were entered in SPSS (version 20.0) and analyzed. Data was summarized as Means ± Standard deviation, frequency and percentage. Chi-Square/ Fishers exact test was used for the comparison of variables and data. The *p*-value of ≤0.05 was considered significant.

RESULTS

A total number of 488 patients, 318 males (65.2%) and 170 (34.8%) females, were evaluated during the duration of study. Mean age of the patients was 47.17 ± 19.21 years. Based on treatment history, 368 (75.4%) and 120 (24.6%) patients were identified as new cases and previously treated patients respectively. Micro-biological diagnosis of pulmonary tuberculosis was achieved in 313 patients (64.1%).

Drug resistant tuberculosis was identified in 74 (15.2%) patients, which was significantly more common in previously treated patients 38 (31.7%) than new patients 36 (9.8%) as shown in table-I. The drug resistant pattern of DR-TB cases and the

results of DST to first line drugs are summarized in table-II and table-III respectively.

MDR TB was the commonest pattern seen accounting for 29 (39.2%) cases of DR-TB. Mono drug resistance was identified in 28 cases (37.8% of DR cases). Fourteen patients (18.9% of DR-TB cases) were diagnosed as Rifampicin resistant on

most common mono resistance 25 (89.3%) of the mono-DR cases).

Rifampicin resistance was present in 38 patients (51.4% of the DR cases) with significantly more cases being previously treated compared to new cases (26 vs 12 cases, *p*-value 0.003).

Table-I: Resistance to anti TB drugs according to treatment history.

	Total cases n=488	New n=368	Previously treated (n=120)	*p*-value
Any drug Resistance detected	74 (15.2 %)	36 (9.8%)	38 (31.7%)	<0.001

Values are presented as number (%)

Table II: Drug resistant pattern among drug resistant TB cases.

Drug resistance pattern	Total n=74	New cases n=36	Previously treated cases (n=38)	*p*-value
Any resistance to Anti-TB drugs	74 (15.2%)	36 (10.84%)	38 (31.66%)	-
Any resistance to Isoniazid	57 (77 %)	28 (77.8%)	29 (76.3%)	0.881
Any resistance to Rifampicinb	38 (51.4 %)	12 (33.3%)	26 (68.4%)	0.003
Any resistance to Ethambutol	9 (12.2 %)	2 (5.5%)	7 (18.4%)	0.08
Any resistance to PZA	23 (31 %)	9 (25%)	14 (36.8%)	0.27
Any resistance to Streptomycin	6 (8.1%)	2 (5.5%)	4 (10.5%)	0.67
Any resistance to Fluoroquinolone	22 (29.7%)	9 (25%)	13 (34.2%)	0.386
Any resistance to Injectables (Amikacin, kanamycin, capreomycin)	3 (4.1%)	1 (2.8%)	2 (5.3%)	1
Mono drug resistance	28 (37.8%)	21 (58.3%)	7 (18.4%)	
Isoniazid	25 (89.3%)	18 (85.7%)	7 (100%)	<0.001
Rifampicinc	1 (3.6%)	1 (4.8%)	-	
Ethambutol	-	-	-	
Pyrazinamide	2 (7.1%)	2 (9.5%)	-	
Rifampicin resistant cases on MTB GeneXpert //RIF only	14 (18.9%)	5 (13.8%)	9 (23.6%)	0.282
Total Multi drug resistant Cases	29 (39.2%)	9 (25%)	20 (52.6%)	0.015
Total poly resistant cases other than MDR	1 (1.4%)	1 (2.7%)	-	0.48
Total XDR	2 (2.7%)	-	2 (5.2%)	0.49

Values are presented as number (%), a: Total number of cases resistant to a drug in any pattern/combination whether mono-resistant, poly-resistant, MDR or XDR etc, b: Cases identified on either DST or gene Xpert/RIF or both, c: Cases proven on DST

MTB Gene-Xpert/RIF alone. Two patients from previously treated category were diagnosed as XDR-TB and one patient as poly resistant TB.

INH resistance was the commonest resistance, identified in total of 57 cases (77% of the DR cases) including mono INH resistance and MDR/XDR cases. INH mono resistance was the

FQ resistance was detected in 22 (29.7%) of the DR-TB cases. Prevalence of FQ resistance among MDR cases was 15 (51.72%) while 5 patients (11.62%) of non-MDR drug resistant TB cases were FQ resistant (fig-1). Resistance to any one of the second line injectables was detected in 3 patients including 2 patients of XDR TB.

DISCUSSION

In this study the drug resistant patterns in patients with pulmonary TB were evaluated with Gene-Xpert/RIF assay in addition to conventional DST. Increase in MDR TB cases globally has made it essential to perform DST to

anti-TB drug resistance estimated the prevalence of resistance to any drug at 11.1% and 25.1% in new and previously treated cases respectively in 2009[9].

INH resistance was the most common drug resistance identified in this study. Total of 57

Table- III: Resistance pattern on DST to first line drugs including streptomycin (n=60).

Drug resistance	Total=60 n(%)	New cases=31 n(%)	Previously treated cases=29, n(%)	p-value
H	25 (41.6)	18 (58)	7 (24.1)	0.007
HRZ	10 (16.6)	4 (12.9)	6 (20.6)	0.50
HR	9 (15)	1 (3.2)	8 (27.5)	0.011
HREZ	4 (6.6)	1 (3.2)	3 (10.3)	0.35
HREZS	4 (6.6)	-	4 (13.7)	0.049
HZ	3 (5)	2 (6.4)	1 (3.4)	1.00
HRE	1 (1.6)	1 (3.2)	-	1.00
HS	1 (1.6)	1 (3.2)	-	1.00
R	1 (1.6)	1 (3.2)	-	1.00
Z	1 (1.6)	1 (3.2)	-	1.00
ZS	1 (1.6)	1 (3.2)	-	1.00

Values are presented as number (%), H: Isoniazid, R: Rifampicin, E: Ethambutol, Z: Pyrazinamide, S: Streptomycin

both first line and second line drugs. Molecular techniques such as MTB Gene-Xpert/RIF assays and Line probe assays (LPA) have improved the diagnosis of tuberculosis and detection of drug resistance. WHO endorsed LPA in 2008 for the rapid diagnosis of TB and detection of rifampicin and INH resistance[6]. GeneXpert MTB/RIF and Geno Type MTBDR plus were recommended in 2011 for diagnosis of TB and MDR-TB in high prevalence countries[7].

Over all 74 patients (15.2%) of the patient enrolled in the study) were found to have drug resistant tuberculosis either on DST or Gene Xpert or both. Resistance to any drug was significantly more common in previously treated cases than new cases (31.7% vs 9.8 %, p-value <0.001). In a large study conducted in Punjab Pakistan, 11.5% isolates of tuberculosis patients were found resistant to at least one drug and the frequency of drug resistance in previously treated TB was found to be 5 times higher than newly diagnosed patients[4]. In Iran 11% of the new TB cases and 40.7% of the re-treatment cases of HIV negative pulmonary tuberculosis were found to be resistant to any TB drug[8]. Global project on

cases (28 new cases and 29 previously treated) were found to have any INH resistance, including 25 cases with mono INH resistance. WHO estimates INH resistance to be present in 8% of the TB patients ranging from 5-11% in different regions[10]. Irfanullah and colleagues had
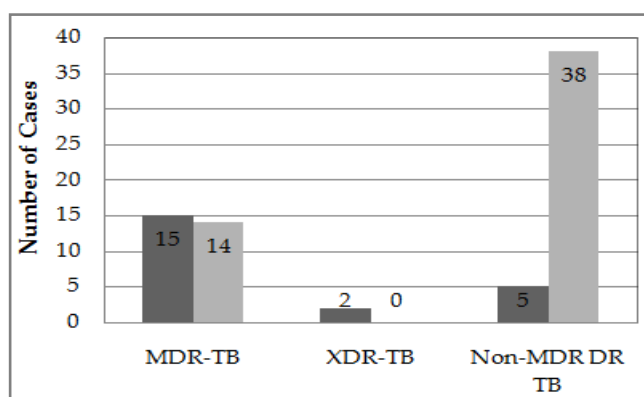
Figure-1: Fluoroquinolone resistance among DR-TB cases.

reported significantly more cases of INH resistance being from previously treated category[4]. High prevalence of INH resistance is concerning especially in new cases. A recent meta-analysis showed that treatment of INH

resistant cases with only first line drugs resulted in increased treatment failure, relapse and acquired multi drug resistance suggesting greater emphasis on rapid detection and effective treatment of INH resistant tuberculosis[5].

Rifampicin resistance was the second most common resistance pattern identified in our study. Over all 38 patients were found to have any resistance to Rifampicin including resistance detected by gene Xpert. 29 cases (39.2% of DR TB cases) among these satisfied the definition of MDR TB, being resistant to both INH and rifampicin, while two cases from previously treated category were diagnosed as XDR-TB case. Significantly more cases of Rifampicin resistance were from the previously treated category as compared to new cases. Rifampicin resistance was detected by Gene Xpert alone in 14 patients (18.9% of DR-TB cases) as their initial and subsequent cultures were either reported negative or contaminated. Cultures of patients with pulmonary tuberculosis may be negative due to variety of reasons including transport and processing problems causing inactivation of tuberculous bacilli, culture contamination, inadequate volume and laboratory or clerical errors. In these cases, treatment may be guided by Gene Xpert result, pending additional testing[11].

In our study, patients with MDR TB were significantly more likely to be previously treated cases. In the study by Irfanullah *et al* 9.3% of culture positive cases in Punjab, Pakistan were diagnosed as MDR TB while the prevalence among drug resistant cases in newly diagnosed and previously treated patients was 4% and 19.7%, respectively[4]. Different socio-economic conditions, living standards and health care delivery systems including TB control programs are the reasons for different prevalence of MDR TB in different regions. In china the prevalence of MDR TB was reported as 2.8% in new cases and 14.7 percent in previously treated cases[12]. In Ethiopia the prevalence of MDR-TB was reported to be 31.4%[13]. Micheletti *et al*. reported prevalence of MDR-TB as 2.2% and 12% in new and

previously treated cases respectively in Brazil[14]. Lack of treatment supervision, poor compliance to medications and ineffective TB control programs are the main causes of MDR TB in previously treated cases while in new cases, the presence of resistance is an indicator of transmission of disease with resistant bacilli[15].

FQ resistance was identified on DST to second line drugs in 15 patients of MDR (51.7%) while among culture positive Non-MDR resistant TB cases, FQ resistant was found in 5 cases (11.6% of cases). Prevalence of FQ resistant TB has been increasing in Pakistan. In a study done in Karachi, Pakistan, the FQ resistance among MDR cases ranged between 54 and 58%, while in non MDR cases, FQ resistance increased from 10.3% in 2010 to 17.1% in 2014[16]. FQ resistance of 52.4% has been reported in MDR TB patients in Peshawar, Pakistan[17]. Prior exposure to FQ has been recognized as a reason for development of FQ resistant TB[18]. TB patients exposed to FQ before the diagnosis of TB may have up to 3-fold increased risk of FQ resistant TB[19]. Increasing FQ resistance in TB patients underscores the importance of stewardship and judicious use of these antimicrobials and raises a concern that empirical treatment of MDR-TB cases with standard MDR treatment regimen in such cases may result in sub optimal management. Molecular DST methods such as Line probe assays are therefore essential for rapid detection of resistance to second line drugs.

We identified 3 patients (4% of the drug resistant TB) resistant to second line injectables, including two cases of XDR TB. Diagnosis of XDR TB has been greatly improved with the advent of molecular methods of detection. WHO recommended use of second line probe DST assay (GenoType MTBDRsl) in 2016 for rapid detection of resistance to second-line fluoroquinolone (FQ) and injectable drugs as well as detecting XDR-TB[20].

Our study had certain limitations. Although we used Gene Xpert MTB/RIF assay, Line probe assay including second line Probe DST was not

available at the time of this study which would have improved the diagnosis of drug resistance tuberculosis. Cases of extra pulmonary tuberculosis were not included in this study. More over the study was done in a single center and sample size was limited, therefore the results of this study cannot be generalized. Large scale multi centre studies and National surveys are therefore required to ascertain the resistance patterns in our country.

CONCLUSION

Drug resistant tuberculosis was frequently identified in previously treated as well as new pulmonary tuberculosis patients. The commonest identified drug resistance patterns are MDR-TB and INH mono-resistance, while fluoroquinolone resistance is prevalent especially among MDR TB cases. These findings underscore the importance of drug susceptibility testing especially molecular methods for rapid detection of drug resistance to both first line and second line drugs for effective treatment in all TB cases.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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