ORIGINAL ARTICLE

DRUG RESISTANT TUBERCULOSIS AMONG PATIENTS IN CHEST UNIT OF MAYO HOSPITAL LAHORE.

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ABSTRACT

Background: Multidrug-resistant tuberculosis (MDR-TB) is an increasing serious global problem and has become all the more frightening over the last decade. Spread of MDR TB is through this repeated unsuccessful treatment and other strong reason is that patient who had no previous history of treatment and is infected with primary MDR TB bacilli from patient who had already developed MDR TB. In 1998, the global TB partners, including the WHO, created "DOTS PLUS" which attempted to address the most glaring deficiencies of DOTS especially regarding treatment of drug resistant TB.

Objectives: To estimate the changing trends in drug resistant tuberculosis in patients visiting tertiary care settings.

Study Settings: This descriptive study was carried out in Pakistan Medical Research Council TB Research Centre in collaboration with Department of Chest Medicine, King Edward Medical University. A total of 1013 cases were included; of which 899 were pulmonary and 114 extra pulmonary.

Material and methods: Primary isolation of *Mycobacterium* was performed on Lowenstein Jensen (LJ) medium. Cultures grown were subjected to drug susceptibility testing on LJ. Medium containing drugs with the following concentrations using standard proportion method: RIF 40.0 µg/ml medium, INH 0.2 µg/ml, Streptomycin 4.0 µg/ml, Ethambutol 2.0 µg/ml. All the tubes were incubated at 37^{o} C +/- 1^{0} C for a minimum of four weeks. Any isolate giving more than 1% growth on the medium containing isoniazid, Ethambutol and rifampicin as compared with control was labeled as resistant strain. The critical proportion for resistance of streptomycin was 10%.

Results: MDR TB was found to be in 16.58% of study subjects. Primary MDR TB was found in 12.83% and acquired was found in 24.18% cases. Mono resistance against Isoniazid, Rifampicin, Ethambutol and streptomycin was found in 6.12%, 6.81%, 2.6% and 2.15% respectively. Forty four percent cases were found to be resistant against at least one of the first line anti TB drugs and 2% cases were found to be resistant against all the four drugs included in this study.

Conclusion: With the induction of "DOTS plus" strategy by TB Control program, MDR-TB is expected to go down. Comparing last five studies being conducted at PMRC TB Research Centre. It does not show any significant increase in drug resistance levels, from which we can conclude that drug resistance is leveling off.

Key Words: *Mycobacterium tuberculosis*, MDR TB, Multi drug resistance, PMRC TB Research Centre,

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Introduction:

Multidrug-resistant tuberculosis (MDR-TB) is an increasing serious global problem. The world Health Organization (WHO) estimates that Mycobacterium tuberculosis caused active disease in 9.15 million people across the globe, killing 1.6 million of them. More people carry the bacillus today (one-third of the world's population) than any other period in history[2].

TB is most common cause of death because; it is one of the most serious infectious diseases with considerable public health problem, due to its high risk of person to person transmission, morbidity and mortality in adults[3]. WHO took an unprecedented step and declared TB to be global emergency[4]. Tuberculosis a well-known bacterial disease for the last 5000 years is still infecting nearly one third of world population with a daily addition of 5000 new cases and loss of two lives every third minute[5].

The strains that are resistant to the two main first line anti TB drugs that form the back bone of short course chemotherapy, isoniazid and rifampicin are known as multi drug resistance tuberculosis. These strains have been found throughout the world and are a significant cause of global TB morbidity and mortality[6,7]. In Pakistan incidence of tuberculosis is estimated as 231 per 100,000 populations and the rising trend of drug resistance is alarming. According to WHO, the amount of drug resistance has been trending upward in many parts of the world and reports show that multi drug resistance tuberculosis is increasing by 3 percent among new patients[8]. The total global burden of multi drug resistance tuberculosis is estimated at almost 490,000 new cases per year, or over 4 percent of all TB cases; an estimated 120,000 of these patients die annually[9].

The problem of drug resistance has become all the more frightening over the last half decade with the emergence of multi drug resistance tuberculosis strains with broad spectrum resistance to both first and second line anti TB drugs. Some of these strains (known as extensively drug resistant tuberculosis) have been found to be resistant to the most effective second line anti TB drugs: fluoroquinolones and one of three parenteral anti TB agents (Kanamycin, Amikacin, Capreomycin)[10].

How drug resistant TB emerges and spreads, is best understood as two interlinked processes. Initially, a patient infected with drug susceptible TB seeks treatment with standard, first line medications. Under proper conditions and assuming only quality assured antibiotics are used, the patient will likely be cured and not relapse[11]. Although patients who fail treatment may have developed some drug resistance, most programs continue to prescribe multiple cycles of first line anti TB therapy. With each iteration of unsuccessful treatment, the number of drugs to which the patient becomes resistant increases, this process is called "amplification of resistance" [12].

Thus in many, the recently recognized increase in global multi drug resistance tuberculosis prevalence, reflects serious deficiencies in both the programmatic approach to treating TB (drug susceptible) and TB treatment delivery at country level. When multi drug resistance tuberculosis strains appear in a given setting, the situation is exacerbated by: constraints on the ability of local practitioners to diagnose drug resistance, largely due to absence of laboratory infrastructure, the lack of a consistent and sufficient supply of quality assured anti TB drugs, and programmatic challenges to delivering TB treatment for the requisite treatment length [13].

In 1998, the global TB partners, including the WHO, created "DOTS PLUS" which attempted to address the most glaring deficiencies of DOTS especially regarding treatment of drug resistant

TB[14]. The present study is based on four years data compiled at Pakistan Medical Research Councils TB Research Centre, King Edward Medical University/ Mayo Hospital, Lahore in which pattern of resistance to individual primary anti tuberculosis drugs among tuberculosis patients have been discussed. Objective of this study is to estimate the changing trends in drug resistant tuberculosis in patients visiting tertiary care settings.

Materials and Methods:

The patients were selected from tuberculosis ward/OPD of Mayo Hospital, Lahore. Original data files of selected patients were retained from the record and total of 1013 cases were analyzed; of which 899 were pulmonary and 114 extra pulmonary (lymph nodes and pus, pleural fluid, gastric aspirate, urine and CSF).All cases showed positive culture for *Mycobacterium tuberculosis*. Only adult patients between the ages of 15 to 60 were included in the study. Previous history of treatment was investigated through medical records and by interviewing the patient and treatment with rifampicin, isoniazid, streptomycin and Ethambutol was investigated. The patients were divided into two groups, one who had no history of taking treatment in the past and the other who had received prior treatment for 4 weeks or more.

The treatment regimens followed these days are rifampicin (RIF), isoniazid (INH), Ethambutol (EMB), and pyrazinamide (PZA) for initial two months followed by RIF, INH and EMB for continuation phase for five months. In selected cases streptomycin is also used.

Culture and drug susceptibility testing were carried out at Pakistan Medical Research Councils, Tuberculosis Research Center, Mayo Hospital, King Edward Medical University, Lahore. Total of 1013 isolates of *Mycobacterium tuberculosis*, grown from various specimens, mostly sputum were tested for drug susceptibility. Only one isolate per patient was included. Primary isolation of mycobacterium was performed on Lowenstein Jensen (LJ) medium. Cultures grown on LJ. medium with PH 6.8 were subjected to drug susceptibility testing on LJ. medium containing drugs with the following concentrations using standard proportion method: RIF 40.0 µg/ml medium, INH 0.2 µg/ml, Streptomycin 4.0 µg/ml, Ethambutol 2.0 µg/ml.

Stock solutions of drugs were made aseptically and added to the medium with PH 6.8 to achieve the desired concentration. A small portion of several colonies was scraped from a culture on Lowenstein Jensen slant and was homogenized using glass beads and vortex mixer. The turbidity of the suspension was adjusted to the McFarland No. 1 turbidity standard. Two dilutions of homogenized bacterial suspension, $10^2$and 10^4were inoculated on each of the drug-containing medium, and on drug free medium which served as a positive growth control.

All the tubes were incubated at 37^o C +/- 1^0 C for a minimum of four weeks. The growth was checked and colonies were counted both on drug containing as well as on control media. The number of colonies on drug-containing medium was compared with those on drug-free medium. The ratio of two was calculated and expressed as percentage. This semi quantitative analysis gave the proportion of mycobacterium population resistant to each drug tested. Any isolate giving more than 1% growth on the medium containing isoniazid, Ethambutol and rifampicin as compared with control was labeled as resistant strain. The critical proportion for resistance of streptomycin was 10%.

Data was further analyzed on the basis of patient's history of previous anti tuberculosis treatment. One group of patients, who had no treatment history, and other group who had definite treatment history for four weeks or more or returned to treatment after having

interrupted treatment for two months or more and patients with doubtful history of treatment were included in the second group. To determine the level of significance between the two groups, chi square (X2) was applied.

Quality Control ATCC H37RV strain of *Mycobacterium tuberculosis* known susceptible to all anti tubercular drugs was used as negative control. External quality assurance is maintained in collaboration with National TB Control Program Pakistan which share Belgium strains for DST and results are compared.

Results:

Data of 1013 culture positive patients with Mycobacterium tuberculosis infection was analyzed and all these had a complete record of susceptibility results.

Table-I shows resistance to five first-line anti tuberculosis drugs; Rifampicin, Isoniazid, Streptomycin and Ethambutol. Multi-drug resistance was seen in 16% cases. The drug resistance pattern from patient with and without history of treatment for various anti tuberculosis drugs showed statistically significant difference. Table II shows resistance to one drug only, Rifampicin and Isoniazid showed maximum resistance. Table III shows the percentage of isolates susceptible to all test drugs along with resistance to one or more anti tuberculosis drugs. Almost 2% patients were resistant to four drugs. The quality control susceptible strains which were run simultaneously with the routine testing yielded satisfactory results.

Table I: Pattern of resistance to individual primary anti tuberculosis drugs among tuberculosis patients. 2009-2012

Drug Resistant To	**All Patients N= 1013 (%)**	**No history of treatment N=678 (66.92%)**	**With history of treatment N=335 (33.07%)**	**P Value**
Isoniazid + Rifampicin. (MDR)	168 16.58%	87 (12.83)	81 (24.18)	< 0.001
Rifampicin	263 25.96%	106 (15.63)	157 (46.86)	<0.001
Isoniazid	246 (24.28)	119 (17.55)	127 (37.91)	<0.001
Streptomycin	217 (21.42)	103 (15.19)	114 (34.03)	<0.001
Ethambutol	136 (13.42)	61 (9.0)	75 (22.39)	<0.001

Table II: Mono Resistance pattern. 2009-2012

Sr. No.	Drugs	Total	%
1.	INH	62	6.12
2.	RIF	69	6.81
3.	ETB	26	2.6
4.	STR	22	2.15

Table III: Susceptible to all the test drugs along with resistance to one and multiple Anti tuberculosis drugs. 2009-2012

Sr. No.	Susceptibility to Drugs	N	%
1.	Susceptible to all test Drugs	565	56
2.	Resistant to Single Drug	179	18
3.	Resistant to Two Drugs	146	14
4.	Resistant to Three Drugs	100	10
5.	Resistant to Four Drugs	23	2

Table IV: Yearly distribution of drug resistance.

Year		2009 N= 342 n (%)	2010 N= 211 n (%)	2011 N = 247 n (%)	2012 N = 213 n (%)	Total 1013 n (%)
Isoniazid + Rifampicin. (MDR)	**No History of treatment.**	30 (8.8)	16 (7.6)	21 (8.5)	20 (9.4)	87 (8.6)
	History of treatment present	28 (8.2)	15 (7.1)	20 (8.1)	18 (8.5)	81 (8.0)
	Total	58 (16.9)	31 (14.7)	41 (16.6)	38 (17.9)	168 (16.6)
Rifampicin	**No History of treatment.**	36 (10.5)	22 (10.4)	26 (10.5)	22 (10.3)	106 (10.5)
	History of treatment present	53 (15.5)	34 (16.1)	38 (15.4)	32 (15.0)	157 (15.5)
	Total	89 (26.0)	56 (26.5)	64 (25.9)	54 (25.3)	263 (16.0)
Isoniazid	**No History of treatment.**	40 (11.7)	22 (10.4)	31 (12.5)	26 (12.2)	119 (11.7)

	History of treatment present	43 (12.6)	24 (11.4)	33 (13.4)	27 (12.7)	127 (12.5)
	Total	83 (24.3)	46 (21.8)	64 (25.9)	53 (24.9)	246 (24.2)
Streptomycin	**No History of treatment.**	35 (10.3)	21(9.9)	24 (9.7)	23 (10.8)	103 (10.2)
	History of treatment present	39 (11.4)	24 (11.4)	27 (10.9)	24 (11.2)	114 (11.3)
	Total	74 (21.6)	45 (21.3)	51 (20.6)	47 (22.0)	217 (21.5)
Ethambutol	**No History of treatment.**	22 (6.5)	12 (5.7)	14 (5.7)	13 (6.1)	61 (6.0)
	History of treatment present	27 (7.9)	15 (7.1)	17 (6.9)	16 (7.5)	75 (7.4)
	Total	49 (14.3)	27 (12.8)	31 (12.6)	29 (13.6)	136 (13.4)

Discussion:

Present study shows data of 4 years i.e 2009 to 2012, and is based on 1013 culture positive cases for Mycobacterium tuberculosis. The overall resistance to drug was high with almost 50% patients showing resistance to one or more drugs. MDR cases, which are almost difficult to treat, were also found in 16% cases. Treatment with Multi drug regimens such as Streptomycin, PAS and isoniazid were introduced with great success and while patients were hospitalized for duration of their therapy (direct observed therapy) very few problems were experienced and cure rates were high[15].In the late 1960s, policies changed and more tuberculosis patients were treated on an ambulatory basis, often without supervision. This led to an increase in drug resistance over time due to poor patient compliance and incorrect use of medication [16].

Previous anti tubercular drug treatment is the largest single risk factor for the presence of multi drug resistance tuberculosis. The rates of resistance in England and Wales in 1995 and 1997 were 6.9-7.2% for isoniazid and 0.9-1.1% for multi-drug resistance tuberculosis in all patients, but 22-33% and 13-17%, respectively, for those patients with a history of prior treatment [17]. In many developing countries, drug resistant tuberculosis is increasing and is a significant threat to tuberculosis control because there are few drugs effective against multi drug resistance tuberculosis [18]. An estimate of drug resistance is therefore, extremely important in the epidemiology and control of tuberculosis. Resistance rates from India are also high where, 25% cases showed resistance to rifampicin, while multi drug resistance tuberculosis was reported to

be 8.1%[19]. A study from Bangladesh reported an overall resistance of 30% to one or more drugs. It showed resistance of 15.8% to isoniazid, 11% to rifampicin 6.9% to streptomycin and 3% to ethambutol in clinically suspected untreated patients [20].

In the present study multi drug resistance tuberculosis cases which are more critical for patient's management were found in 16.5% cases. If we look at the most effective drugs individually, rifampicin and isoniazid resistance was very high i.e. one fourth of patients had resistance to these drugs, followed by resistance to streptomycin indicating the frequent use of this drug in treating tuberculosis. Resistance to ethambutol was comparatively lower. In Pakistan due to low literacy rate it is difficult to establish past history of anti-tuberculosis treatment as patients change physicians and institutions quite frequently. Facility for computerized or central record keeping is also not available in most institutions in Pakistan as it is a retrospective analysis; therefore, one has to rely on patient's history available in our record. Though we tried our level best to get an accurate past history of taking anti tuberculosis treatment but still many patients who are in the category of no history of treatment may actually be the treated cases with acquired drug resistance. However, infection in fresh untreated cases due to strains already resistant cannot be ruled out. As samples were collected from only one site, it is true that results cannot be generalized for entire country however Mayo hospital being a very old tertiary care setting, patients usually come from various districts of Punjab therefore results to some extent could reflect the situation in Punjab.

We compared our findings with previous studies carried out in the same area. Siddiqi et al in 1976 reported very low resistance to isoniazid, streptomycin and para amino salicylic acid. Rifampicin was not used in those days [21]. Second study done by same group in 1981 reported more than 1000 patients and showed emerging low resistance to rifampicin and isoniazid [22]. With the induction of rifampicin, ethambutol and pyrazinamide regimen for the treatment of tuberculosis, the resistance pattern changed and was reflected in a follow-up study published by Aziz et al in 1989, reporting resistance to five drugs including pyrazinamide. Even at that time one third of patients showed resistance to one or more drugs [23]. Iqbal et al in 2003, 2005 and 2008 reported similar pattern of resistance, with resistance of 27% in rifampicin, 25 % in isoniazid, 23% in streptomycin, 14% in ethambutol and 30% in pyrazinamide[24-26]. On comparison of these studies MDR-TB cases showed an upward trend from 3.1% in 1981, 7.7% in 1989, 23% in 2003 and 27% in 2008. Another study from Armed Forces Institute of Pathology, Rawalpindi showed MDR TB of 30% in treated cases with 2% increase from their previous report[27].While, a study from Rawalpindi, showed multi drug resistance of 16%[28]. A study from Sindh showed 25% MDR-TB in treated cases which, is in agreement with present study[29].

Today, standard treatment for TB patients lasts six months and the regimen for most patients with MDR-TB takes 20 months minimum. Treatment for MDR-TB is costly and can have serious side-effects. While the number of cases of MDR-TB notified in the 27 high MDR-TB burden countries is increasing and reached almost 60,000 worldwide in 2011, this only one in five (19%) of the notified TB patients estimated to have MDR-TB. Globally, 3.7% of new cases and 20% of previously treated cases are estimated to have MDR-TB[8].

WHO has recommended some principles for managing cases of multi drug resistance tuberculosis in developing countries where, such cases should only be treated by physicians experienced in treating complex cases, in close collaboration with national and regional

mycobacteriology services utilizing drug susceptibility data[30]. As suggested earlier TB Control Program has started “DOTS Plus” schemes and have provided GeneXperts which has provided rapid MDR diagnosis within 2 hours. This will rapidly improve drug susceptibility testing facilities in the laboratories across the country and will provide prompt management of MDR-TB patients.

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