Sensitivity Pattern of Mycobacterium Tuberculosis at Lahore, (Pakistan).

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This study was designed to determine the incidence of resistance to four first line anti tuberculosis drugs, Rifampicin, INH, Ethambutol and Streptomycin and compare it with other local and international studies. We studied 100 patients of Pulmonary tuberculous (68 men and 32 women). Sixty-one (61) were newly diagnosed and 39 patients and anti-tuberculosis treatment previously. Sensitivity tests were performed on the sputa according to the proportion method. It revealed that 36% of the bacterial isolates were resistant to one or more drugs. Fourteen percent isolates had resistance to one drug, 13% to two drugs, 5% to three drugs and 4% to all the four drugs. Multidrug resistance (MDR) defined, as resistance to both rifampicin and isoniazid was present in 11% of the isolates. Overall resistance to individual drugs was isoniazid 25%, streptomycin 19%, rifampicin 15% and ethambutol 12%. Previous exposure to anti tuberculosis drugs emerged as the most important factor associated with drugs resistance especially MDR-TB. (p value < 0.001) Comparison with global surveillance report revealed that the incidence is well above the median in that study. Our results are comparable to those reported in that report from Delhi region of India, our neighboring country. Most alarming is the increasing resistance to rifampicin in this region. It has increased from reported 2% in 1993 to 15% in this study. Incidence of drug resistance is high in this region and it is increasing at an alarming rate. Ineffective treatment is the single most important factor associated with emergence of drug resistance. Implementation of effective tuberculosis control programme is urgently required and surveillance of drug resistance should be continued to monitor the scale and nature of drug resistance as well as the success of control measures.

Key Words. MDR, Anti tuberculosis Treatment, DOTS, Drug resistant TB.

History of antituberculosis drug resistance is almost as old as the history of drugs themselves. Soon after the discovery of streptomycin, clinical trials conducted to evaluate its efficacy in tuberculosis in Great Britain by the Medical and Research Council[1] highlighted this problem of emergence of resistant strains when single drug was used. However effective combination therapy evolved with the discovery of other drugs. Surveillance of drug resistance in developed countries with good tuberculosis control programs showed declining proportion of drug resistance organisms after the introduction of antituberculosis drugs (during 1950-86). This led to loss of interest in the subject among the leaders in clinical medicine, public health and biomedical research despite the fact that the problem of resistance was quite substantial in developing countries with poor TB control programs. Then world saw the reversal of this downward trend in the incidence of tuberculosis to the extent that WHO had to declare tuberculosis as a global emergency[2].

In its 1995 report, WHO estimated that as many as 100 million people now may be infected with drug resistant strains, some of which do not respond to any known antituberculosis drug. These drug resistant strains are only created by poor or incomplete treatment which is common in poorly funded TB control programs. If infectious persons are not cured, new infections will continue at a rate of one per second. As the faulty TB programs favour the spread of drug resistant strains, we are coming closer to return of the days when we will once again have no treatment for tuberculosis except isolation in sanitoria[3].

Situation in Pakistan is even more serious. Although DOTS has been adopted as TB control strategy the population covered is still very small. It is estimated that about 1.5 million people out of a total population of 130 million suffer from TB and more than 210,000 new cases occur each year. Approximately 25% of new cases are ever diagnosed and only a fraction of doctors know how to prescribe effective treatment. Drug resistant strains are likely increasing at an alarming rate. Therefore, it is clear that Pakistan has been losing the war against tuberculosis[4].

Drug resistant tuberculosis had to appear in USA and Europe[5-7] to clinch the international attention once again. WHO and IUAT & LD launched a project on antituberculosis drug resistance between 1994-97, in which resistance to 4 first line drugs, rifampicin, isoniazid, ethambutol and streptomycin, (RHES) was studied in 35 countries or regions[8]. Pakistan was not part of that study. Drug susceptibility tests are being routinely carried out in our Institute. We decided to see the drug resistance to same four drugs, (RHES) during 1997-98 in our patients and compare it to those in that report and other international & local studies.

Methods:

This study included 100 patients suffering from pulmonary Tuberculosis of either sex or age group who were admitted in Institute of Chest Medicine, Mayo Hospital Lahore

during 1997-98. The culture and sensitivity was done at Tuberculosis Research Center of Pakistan Medical Research Council (PMRC) which is situated in the premises of institute and affiliated with it.

Three successive morning sputum specimens from each patient were processed for isolation of mycobacteria by concentration method. The procedure adopted was as recommended by Annie L. Vestal B.S., C.D.C., USA[9]. The specimens were homogenized and decontaminated with sodium hydroxide 4%, treated with buffer solution and then centrifuged for concentration. The sediment was inoculated on modified Lowenstein Jensen (LJ) medium and smear prepared for staining and microscopic examination. The smears were stained by Ziehl Neelsons' method and examined (at least 200 oil immersion fields) to report for presence of acid fast bacilli. The inoculated L.J. media slopes were incubated at 37 ^{0} C for minimum of 6 weeks. All the isolated acid-fast cultures were checked for purity, colonial characteristics, rate of growth and subjected to biochemical tests for identification[10].

The pure cultures of M. tuberculosis thus obtained were exposed to drug susceptibility testing according to the proportion method as recommended by Cannetti et al[11]. Only one standard concentration of the filtered stock solution of each of the following drugs was incorporated into the L.J medium before inspissation. The amount of drug used per ml of L.J. medium was isoniazid 0.2 mcg, ethambutol 2.0 mcg, rifampicin 40.0 mcg and streptomycin 4.0 mcg. A standard suspension of the test strain of M. tuberculosis was prepared comparable to Macfarland standard No. 1. After making a serial tenfold dilution of bacterial suspension, two dilutions 10^{3} x 10^{5} were inoculated on a set of two L.J slopes containing drug and a set of two L.J. slopes without any drug as a control. The growth was compared after a minimum of 4 weeks incubation at 37 ^{0} C [12]. The growth was checked and colonies counted on the control as well as drug containing media. The average number of colonies obtained for control slopes indicated the number of culturable particles contained in the inoculum. It was compared with the drug containing slopes. The ratio of the two indicated the proportion of resistant bacilli existing in the test strain[13]. Below certain proportion (the critical proportion) the strain was classified as sensitive and above as resistant and was reported in percentage. The critical proportion (CP) for drugs used was 1% for isoniazid, ethambutol, rifampicin and 10% for streptomycin.

Results:

Characteristics of patients and their disease is given in Table1. Sensitivity pattern of Mycobacterium tuberculosis and relative importance of previous history of ATT is shown in Table 2. Overall, 36% patients had resistance to one or more drugs. Multidrug resistance (MDR), defined as resistance to at least rifampicin and isoniazid was present in 11%. There were 61 newly diagnosed patients and 39 patients had previous history of antituberculosis treatment. Sensitivity pattern in the two groups is presented in this table. Resistance to one or more drugs was present in 56.4% Mycobacterial isolates from patients in treated group (Acquired resistance) and 23% isolates from patients in previously untreated group (Primary resistance). Resistance to individual drugs(Table 3)

Table 1 (n=100)

Male 68, Female 32, Male to Female ratio: 2.12 : 1			
Age range 12-80 years			
Mean age 41.39 $\pm$ 17.54 years			
72% patients were in economically productive Age group i.e., 21-60 years			
History of contact with TB patient		20%	
Family History of TB		17%	
Sputum smear positive for AFB			18%
Type of patients			
Newly Diagnosed		61%	
Previously treated patients			39%
Defaulter	17%		
Relapse	15%		
Failure	2%		
On ATT	5%		
Radiological extent of disease			
Minimal	10%		
Moderately advanced	44%		
Far advanced	46%		
Cavitary Disease	58%		
Non Cavitary Disease	42%		

Table 2 Previous History of ATT and Drug Resistance

	No history of ATT	History of ATT	Total
Fully sensitive	47(77%)	17(43.6%)	64
Resistance to one or more drugs	14(23%)	22(56.4%)	36
Resistance to one drug	8(13.1%)	6(15.4%)	14
Resistance to two drugs	5(8.2%)	8(20.5%)	13
Resistance to three drugs	1(1.6%)	4(10.3%)	5
Resistance to four drugs	0	4(10.3%)	4
Total	61(100%)	39(100%)	100

Table 3 Pattern of resistance for individual drugs

Drug	Overall Resistance (n=100)	Primary resistance (n= 61)	Secondary resistance (n=39)	*P* value
INH	25 (25%)	8 (13.1%)	17 (43.6%)	<0.001
Streptomycin	19 (19%)	6 (9.8%)	13 (33.3%)	<0.01
Ethambutol	12 (12%)	4 (6.6%)	8 (20.5%)	>0.05
Rifampicin	15 (15%)	3 (4.9%)	12 (30.8%)	<0.001

N.B. p value<0.05=Significant>0.05=Not significant

Once again, in the treated patients, much higher resistance was found. In cases of MDR TB, ten out of 11 patients (91%) had previous history of anti tuberculosis treatment. Considering total number in each group, amongst the 61 patients with no previous history of antituberculosis treatment, 1 patient (1.64%) had MDR (Primary Multidrug resistance) while out of 39 patients exposed to antituberculosis treatment previously, 10 patients (25.64%) had MDR (Acquired Multidrug resistance).

Discussion:

This study shows that drug resistance to antituberculosis drugs prevalent in our society but the problem is not limited to our population rather it is a global phenomenon. It threatens the success of tuberculosis control programs worldwide. Drug resistance is found in both the developed nations like USA, France, England and Wales, Australia & Spain as well as developing ones like Cuba, India, Latvia etc. Pablos-Mendez et al (1998)[8] has reported the results of a global project on antituberculosis drug resistance surveillance between 1994-97 in which resistance to 4 first line drugs, rifampicin, INH, streptomycin and ethambutol was studied. A comparison of prevalence of combined drug resistance in the two studies(Table 4).

Table 4.Comparison of combined prevelance of drug resistance

	Global Surveillance Report				This Study
	Min	Max	Median	India	
Resistance to any drug	2.3	42.4	12.6	32.4	36.0
Resistance to H	2.3	39	9.2	28.8	25
Resistance to R	0	23	2.7	14	15
Resistance to E	0	9.4	1.5	7	12
Resistance to S	0	35.1	0.9	18.1	19
Resistance to HERS	0	7	0.6	3.5	4
Resistance to >1 drugs	1	34.7	5	21.5	22
MDR	0	22.1	2.2	13.3	11

H= Isoniazid, R=Rifampicin, E: Ethambutol, S=Streptomycin, MDR= Multidrug resistance

Drug resistance is much more prevalent in our population when compared with the median in that project. However the results from Delhi region of India reported in that project shows remarkable similarities. Previous exposure to antituberculosis drugs emerged as the most important factor associated with drug resistance both in present study as well as global project (Table 5 & 6). Resistance to INH was most prevalent, followed by streptomycin as shown in Table 7.

Table 5 Comparison of prevalence of Acquired drug resistance

	Global Surveillance Report			This study
	Min	Max.	Median	
Resistance to any drug	5.3	100.0	36.0	56.4
Resistance to 1 drug	4.5	65.2	12.2	15.4
Resistance to 2 drugs	0	30.3	9.7	20.5
Resistance to 3 drugs	0	33.8	5.4	10.3
Resistance to 4 drugs	0	17.1	4.4	10.3
Resistance to>1 drugs	0	68.9	19.9	41.1
MDR	0	54.4	13.0	25.6

Table 6.Comparison of prevalence of Primary drug resistance

	Global Surveillance Report			This study
	Min	Max	Median	
Resistance to any drug	2.0	40.6	9.9	23.0
Resistance to 1 drug	1.0	25.7	6.6	13.1
Resistance to 2 drugs	0	12.4	2.5	8.2
Resistance to 3 drugs	0	9.5	0.6	1.6
Resistance to 4 drugs	0	4.6	0.2	0.
Resistance to >1 drugs	0.2	26.5	3.8	9.8
MDR	0	14.4	1.4	1.6

Table 7. Comparison of Primary resistance in various drugs

	Global Surveillance Report			This study
	Min	Max.	Median	
Isoniazid	1.5	31.7	7.3	13.1
Rifampicin	0	16.8	1.8	4.9
Ethambutol	0	9.9	1.0	6.6
Streptomycin	0.3	28.0	6.5	9.8

A number of studies[14-19] done in recent past has shown that the Drug resistance is a global problem and after the initial decline, it has geared up again. It is true for Pakistan as well, In 1967, 87% of previously treated patients were resistant to one or more drugs which decreased to 31.6% in 1989[20].

Most alarming is the increasing resistance to rifampicin. S Rasul et al[21] reported a 2% resistance to rifampicin. Only two years later, in 1995 resistance to rifampicin was found in 5.4% of 149 isolates[22]. In present study, 15% of the isolates showed resistance to rifampicin. Significantly, all these studies were conducted at Lahore. Rifampicin is the cornerstone of any anti tuberculosis regimen and increasing resistance to rifampicin might

jeopardize the efforts to control tuberculosis. In treated group, resistance to rifampicin was 30.8% and these patients are transmitting disease in the community as shown by 4.9% primary resistance. This trend is to be checked if we want to control tuberculosis.

Drug resistance is significantly higher in maltreated patients. Patients are always blamed for non-compliance and in fact one third of patients can be expected to be non-compliant[23]. Non compliance on the part of doctors also pose a big problem. In their study, "Family doctors & Tuberculosis control", Shamshad Rasul et al (1995)[24] interviewed two groups of doctors, 87 practicing in the community and 25 appearing in Punjab Public Service Commission (P.P.S.C.) for the post of medical officer. When asked to prescribe treatment for a TB patient, only four from each group (4.6% of general practitioners and 16% of those appearing in P.P.S.C.) could prescribe adequate regimen. Obviously the situation is for from satisfactory in country where the tuberculosis is so prevalent. Global tuberculosis control program of WHO held a workshop on "tuberculosis control and medical school" in Rome in 1997. It had looked into this aspect and recommended that the medical school should provide every graduate the knowledge, skill and attitude essential to the management of tuberculosis in the patients and in the community as a whole. The results of educational process should be adequately assessed and evaluated before the medical student is allowed to graduate as a doctor[25].

Drug resistance is a measure of medical malpractice. The single most important measure against drug resistance is to ensure that it dose not happen. This is achieved by making it certain that every patient complete a full course of treatment. Effective therapy not only cures the patients it also prevents the spread of tuberculosis and even more importantly prevents the development of Drug resistant strains. DOTS strategy is widely recommended by all the quarters concerned. But it will work only when it is implemented in its true sense and spirit. That is:

- Political will of governments to implement the TB control program,
- uninterrupted supply of good quality anti tuberculosis drugs,
- Availability of necessary expertise to diagnose & prescribe right treatment,

Only then one can be able to directly observe the therapy and ensure the compliance of patients.

WHO and IUATLD are continuing its global project on antituberculous drug resistance surveillance and its second report has been published[26]. It is recommended that surveillance of Drug resistance should be continued as it is essential to determine the current scale and nature of Drug resistance problem as well as to define the correct solution. It also measures the success of a tuberculosis control program.

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