Study of Initial and Acquired Drug Resistance of *Mycobacterium Tuberculosis* in Pulmonary Tuberculosis

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Research Article

Abstract: We studied the resistance pattern of *Mycobacterium tuberculosis* in Category I and Category II Group of pulmonary tuberculosis patients. **Materials and methods**: 79 culture positive patients in Category I and 75 in Category II were subjected to antibiotic sensitivity testing using drug incorporated LJ medium. The drugs tested were primary drugs i.e Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, Streptomycin and secondary drugs i.e. Ciprofloxacin, Ofloxacin, Moxifloxacin (Quinolones) Amikacin and Cycloserine. Resistance was intrepretated using MIC method (all drugs) and resistance ratio method (Streptomycin).**Results:** 48.11% showed resistance to one or more primary drug in Category II as against, 12% in Category I. Maximum resistance was shown to INH in Category II (30.37%) followed by Rifampicin (29.11%) This was again significantly higher than their resistance in Category I. Resistance was not observed to Pyrazinamide. When further pattern of drug resistance was analyzed, resistance to single drug was maximum followed by resistance to two drugs and then three drugs. All the strains in Category I and II showed significantly high resistance to Quinolones. Of the 9 MDR strains in Category II,

- 7 were resistant to Quinolones.
- 3 were resistant to Cycloserine.
- 2 were resistant to Amikacin.

While in Category I, only 1 MDR strain was isolated which was also resistant to Quinolones. Resistance to Quinolones remained high irrespective of whether the strains remained sensitive or resistant to primary drugs. Primary drugs still retain the "essence" of treatment Indiscriminate use of Quinolones must be avoided.

Keywords: Category I and II, Initial Drug Resistance, Acquired drug resistance, MDR Multidrug resistance.

Introduction

Tuberculosis is the most common cause of death due to a single infectious agent worldwide in adults (1).It is estimated that about 1/3rd of the population is infected and another 300 million will be infected in the next decade (2). Resistance could be either Initial or Acquired drug resistance. A high Initial resistance rate resulted from an inefficient National Tuberculosis Programme in the past but a high Acquired resistance is more likely to represent a poorly functioning National Tuberculosis Control Programme currently in operation (3). Higher the number of excretors of resistant bacilli after treatment, then higher the risk that these resistant bacilli are being transmitted to healthy individuals. New patients although not previously treated will be as a matter of course be carriers of resistant bacilli. This is "Primary drug resistance" which is both a consequence of Acquired resistance and an altered reflection of it (4). **"Thus the rate of Primary resistance in comparison to Acquired resistance is an excellent epidemiological indicator for long term surveillance of anti tuberculosis chemotherapy as it is really, applied in the community"** (4). With this background, the present study was planned to compare the resistance pattern between Category I and Category II group of patients. As second line drugs are to be used in Category II (Drug resistant tuberculosis) group of patients, resistance to these drugs i.e. Quinolone, (Ciprofloxacin, Ofloxacin, Moxifloxacin), Aminoglycoside (Amikacin) and Cycloserine were also determined and compared with that in Category I group of patients.

Materials and Methods

The Present study was carried out in the Department of Microbiology in collaboration with District Tuberculosis Center at Shri Chhatrapati Shivaji Maharaj Sarvopachar Rugnalaya, Solapur, during the time period of 1 year.

The sample size of present study was calculated according to the formula

$$N = \frac{(Z)^2\ 1 - \alpha/_2 \ x\ p^{(1-P)}}{} \qquad (5)$$

200 sputum samples each in category I and category II were collected, of which

M tuberculosis was isolated in 75 samples in Category I and in 79 samples in Category II. The isolates were identified depending on colony morphology, rate of growth, pigment production, niacin test, nitrate reduction test, sensitivity to paranitro benzoic acid. Antimycobacterial sensitivity testing was carried out by incorporating the various drugs in LJ medium. The sensitivity testing was done by using.

a) Minimum Inhibitory Concentration (MIC) method for INH, Rifampicin, Ethambutol, Ciprofloxacin, Ofloxacin, Moxifloxacin, Amikacin and Cycloserine and
b) Resistance Ratio (RR) method for Streptomycin (6).

The inoculum was standardized and a loopful of the suspension was spread on the surface of each LJ slope for the sensitivity test. As a control, a drug free slope was set up for each strain tested. The standard sensitive strain $H_{37}Rv$ obtained form National Tuberculosis Center, Bangalore was also treated in each set of tests. The inoculated slopes were incubated at 37^0C and the media were observed on every alternate day. . For all tests 'growth' was defined as the presence of 20 or more colonies on the slope.

For Streptomycin, the resistance ratio was calculated as

$$\text{Resistance ratio (RR)} = \frac{\text{MIC of test strain}}{\text{MIC of H37Rv strain}}$$

Definition of resistance

Sensitive – RR of less than 2
Resistance – RR of greater than 8
Doubtful – RR of 4

Drugs	Criteria for resistance
Isoniazid	MIC ≤ 1 mg/l
Rifampicin	MIC ≤ 128 mg/l
Ethambutol	MIC ≤ 8 mg / l

MIC for Quinolones and Amikacin and Cycloserine

To determine the MIC for Quinolones and Amikacin and Cycloserine, we prepared the stock solution of these drugs. Various dilutions from these stock solutions were made. These dilutions were incorporated in LJ media to get final concentrations. Standardized inoculum of reference strain H37Rv was inoculated on these drug containing slopes to find out the MIC.

The MIC of each drug was recorded as.

Drugs	MIC value (μg /ml)
Ciprofloxacin	0.5
Moxifloxacin	0.25
Ofloxacin	0.5
Amikacin	8
Cycloserine	20

Results

75 strains (37.5%) of *M tuberculosis* were isolated in Category I , while 79 strains (39.5%.) were isolated in Category II. Antimycobacterial sensitivity testing showed higher resistance to one or more primary drugs in Category II (48.11%) over Category I (12%) ($P < 0.001$). (Table No.1). Isolates from Category II cases showed higher resistance to second line drugs like Quinolone (53.16%) Cycloserine (13.92%) Amikacin (6.32%) over Category I isolate. (Table No.1) Analysis of resistance to individual drug showed that resistance to all primary drugs was higher in Category II over Category I Amongst them resistance to INH (30.37%), Rifampicin (29.11%) were significantly more ($p<0.001$) than Category I (5.33% for INH and 9.33% for Rifampicin respectively). (Table No.2) No resistance was observed to Pyrazinamide .Combination of drugs holds the key to success of treatment of tuberculosis. There by, single drug resistance and multidrug resistance were analyzed. Single drug resistance in Category II cases was to INH (15.18%) followed by Rifampicin (13.92%) where as in Category I, Rifampicin (6.66%) was leading. (Table No. 3) Similarly combination of two drugs showed maximum resistance to HR combination (5.06%) in Category II (Table No. 3). Three drug resistance pattern showed maximum resistance to HRS (5.06%) in Category II. (Table No. 3). Four drug resistance pattern showed only one strain resistant to all four drugs in Category II and none in Category I. (Table No. 3). Quinolones showed maximum resistance pattern in both Category I and Category II strains among second line drugs. Both 3 drugs and 2 drugs combination of Quinolones showed high resistance pattern in Category II(both 17.72%) while only 2 drug combination showed high resistance in Category I (9.33%). Ofloxacin showed maximum single drug resistance (11.39%) in Category II while ciprofloxacin showed maximum single drug resistance in Category I(Table No.4). Among MDR strains,77.77% in Category II were resistant to Quinolones,33.33% to Cycloserine and 22.22% to Amikacin,1 strain was found in Category I which was also resistant to Quinolones. Among strains that were sensitive to all primary drugs,43.9% in Category II and 18.46% in Category I were resistant to Quinolones,while in strains showing resistance to primary drugs 60.52% in Category II and 50% in Category I were resistant to Quinolones.(Table.No 5)

Table 1: Distribution of drug resistant strains of *M tuberculosis* to individual antimycobacterial drug

No. of strains	Category I n = 75	Category II n = 79	p value*
Resistant to one or more primary drugs	9 (12%)	38 (48.11%)	< 0.001
Resistant to one or more Quinolones	18(24%)	42(53.16%)	< 0.001
Resistant to Amikacin	3(4%)	5 (6.32%)	> 0.05
Resistant to Cycloserine	10(13.33%)	11 (13.92%)	> 0.05
Bot primary and secondary , Resistant to one or more drugs	24(32%)	57 (72.15%)	< 0.001

P values <0.001 indicates highly significant, p value > 0.05 indicates insignificant

Table 2: Distribution of drug resistant strains of *M tuberculosis* to individual primary antimycobacterial agents

Drugs	Resistant strains to drugs		P value
	Category I	Category II	
	n = 75	n = 79	
Isoniazid INH (H)	4 (5.33%)	24 (30.37%)	< 0.001
Rifampicin (R)	7(9.33%)	23 (29.11%)	< 0.001
Ethambutol (E)	1(1.33%)	2 (2.53%)	> 0.05
Streptomycin (S)	2 (2.66%)	10 (12.65%)	< 0.05
Pyrazinamide (Z)	Nil	Nil	

P values < 0.001 indicates highly significant, p value > 0.05 indicates insignificant. < 0.05 indicates significant.

Table 3: Resistance to single or multiple drugs among primary drugs

One Drug			Two Drugs			Three Drugs			Four Drugs		
	Category I(%)	Category II(%)		Category I(%)	Category II(%)		Category I(%)	Category II(%)		Category I	Category II(%)
H	1(1.33)	12(15.18)	HR	Nil	4(5.06)	HRS	1 (1.33)	4(5.06)	HRSE	Nil	1(1.26)
R	5(6.66)	11 (13.92)	HS	1(1.33)	2 (2.53)	HRE	Nil	1(1.26)	Total	Nil	1(1.26)
S	Nil	1(1.26)	RS	1(1.33)	2 (2.53)	Total	1(1.33)	5(6.32)			
E	Nil	Nil	Total	2(2.66)	8(10.12)						
Z	Nil	Nil									
Total	6(8)	24(30.37)									

Table 4: Distribution of drug resistant strains of *Mycobacterium tuberculosis* in Quinolones

No. of Strains Resistant	Category I n = 75	Category II n = 79	'p' value
All Quinolones Ciprofloxacin + Ofloxacin + Moxifloxacin	1 (1.33%)	14(17.72%)	< 0.001
Two Quinolones Ciprofloxacin + Ofloxacin	7 (9.33%)	14 (17.72%)	> 0.05
Single Quinolone			
Ciprofloxacin	6 (8%)	3 (3.79%)	>0.05
Ofloxacin	1 (1.33%)	9(11.39%)	<0.05
Moxifloxacin	1 (1.33%)	1 (1.26%)	Nil

Table 5: Comparison of resistant rates to second line drugs among strains of *M tuberculosis*

	Quinolones		Cycloserine		Amikacin	
	Category I	Category II	Category I	Category II	Category I	Category II
MDR strains H+R n = 1in Category I, n = 9 in Category II.	1 (100%)	7 (77.77%)	0	3 (33.33%)	0	2 (22.22%)
Sensitive to all primary drugs (HRSE) n = 65 in Category I n = 41 in Category II	12 (18.46%)	18 (43.90%)	7 (10.76%)	5 (12.19%)	3 (4.61%)	3 (7.31%)
Resistant to all primary drugs (HRSE) . n = 10 in Category I , n = 38 in Category II.	5 (50%)	23 (60.52%)	3 (30%)	6 (15.78%)	1 (10%)	3 (7.89%)

Discussion

The level of drug resistance in a country is known to provide an epidemiological indicator to assess the extent of resistant bacterial transmission as well as success of National Control Programme. With this background, the present study was planned to assess the Initial and Acquired drug resistance in *Mycobacterium tuberculosis* isolated from patients attending Shri.Chhtrapati Shivaji Maharaj Sarwopachar Rugnalaya. The sample size included 200 cases in each of Category I and Category II over a period of one year. In our study, isolation of *Mycobacterium tuberculosis* in Category I and Category II was 37.5% and 39.5% respectively. We observed that resistance to one or more primary drugs was significantly high (p <0.001) in Category II (48.11%) over Category I (12%)(Table 1) Hirano *et al* in 1996 has reported very low resistance from Japan that i.e. 5.6% Initial to one or more drugs and 27.8% Acquired resistance to one or more drugs (7)In year 2002, Malhotra *et al* have reported Primary and Acquired resistance to be 15.9% and 43.5% to one or more drugs respectively.(8).In our study, maximum resistance was observed to INH in Category II (30.37%) followed by Rifampicin (29.11%)(Table 2). Resistance to INH and Rifampicin in Category II cases was significantly (p<0.001) higher than the resistance observed in Category I cases (Table 2).In 1992, Jain *et al*

had reported that Initial and Acquired resistance to INH was higher than Rifampicin i.e. Initial drug resistance to INH was 18.5% and Acquired drug resistance was 50.7% while Initial drug resistance to Rifampicin was 0.6% and Acquired drug resistance to Rifampicin was 33.3% (9) Malhotra *et al* have reported that Acquired resistance to INH is 39.7%.over Initial resistance of 13.6% whereas Rifampicin showed Acquired resistance of 28.2% as against 6.8% of Initial resistance (8). As combination of Isoniazid + Rifampicin (HR) resistance is used for defining MDR strains, we came across very low rate of MDR strains. MDR reported from Kenya at one end is 0% while at Ivanovo territory of Russia at the other end is 100% (10).We had observed only one strain 1.26% in Category II which was resistant to all four drugs. There was not a single strain in Category I, which showed resistance to all the four drugs. Our study however corresponded to the findings of Sophia V *et al* who reported 0.4% Initial drug resistance to four drugs (11). Among second line drugs, fluorinated quinolones are attractive candidates for the treatment of chronic mycobacterial infections when resistance emerged to conventional antituberculosis drugs (12) Thus, we evaluated resistance pattern to quinolones and observed that as a single drug Oflaxocin showed maximum resistance in Category II (11.39%) which was significantly higher than that in Category I (Table 4). Surprisingly resistance to Ciprofloxacin in Category I. (8%) was more than that in Category II (3.79 %)(Table 4). Grimaldo *et al* have reported a comparatively higher resistance to Quionolones with single drug resistance to Ofloxacin as 33.9%, which was more than that to Cipro (13) The combinations of Ciprofloxacin + Ofloxacin + Moxifloxacin and Ciprofloxacin + Ofloxacin showed the same resistance pattern ie 17.72% in Category II patients(Table 4). This revealed that there was cross resistance between the two Quinolones, Ciprofloxacin and Ofloxacin while addition of Moxifloxacin did not effect the resistance pattern. Grimaldo *et al* have also reported a very high cross resistance in between Quinolones (Cipro + Oflox) ie 53.2%.Ofloxacin (12.9%) (13). We isolated 9 MDR strains in Category II of which 7 ie 77.77% were resistant to Quinolones. Also 22.22% and 33.33% of these MDR strains were resistant to Amikacin and Cycloserine respectively. (Table 5) During the period 1995-2000, Grimaldo *et al* observed 51.4% of MDR strains resistant to Quinolones. There is an increase in resistance of MDR *M. tuberculosis* strains, to Quinolones from 10.3% - 24.0% in 1989-1994, to 51.4% observed during 1995-2000 (13). Table 5 showed that resistant to Quinolones is not related to resistance to primarydrugs. It also implies that Quinolone resistant mutants are circulating at a considerably high frequency in the community. This corresponds with the study of Casal *et al* and Grimaldo *et al*(14,13)Thus, the introduction of Quinolones has already exerted a selection pressure for the emergence of *Mycobacterium tuberculosis* strains resistant to Quinolones over these years. Quinolones were extensively used for the treatment of gastrointestinal tract infections, septicemia , respiratory tract infections, urinary tract infections, etc. prior to their use in tuberculosis. This use in patients harbouring *M. tuberculosis* might have induced resistance in these strains. Also the high cross resistance rates between these groups of drugs makes them increasingly ineffective as second line drugs. Thus if they are to be used, they must be used in judicious combination with other drugs to which *M.tuberculosis* are sensitive. In order to ensure the effectiveness of the DOTS- Plus programme , use of these second line drugs for the treatment of other common infections must be curtailed and second element of DOTS-plus strategy that is culture and susceptibility testing of all the isolated strains must be emphasized. There was no significant difference in the resistance patterns to Amikacin and Cycloserine between susceptible and resistant strains in Category I and Category II (Table 5). We did not come across any literature comparing the role of Amikacin and Cycloserine in Category I and Category II cases.

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