SENSITIVITY PATTERN OF *MYCOBACTERIUM TUBERCULOSIS* AGAINST FIVE ANTITUBERCULOSIS DRUGS.

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ABSTRACT:

Bacterial activity of 98 strains of M. tuberculosis was conducted against major anti-tuberculosis drugs, streptomycin, INH, ethambutol, rifampicin & pyrazinamide. Sixty four isolates were isolated from newly diagnosed cases of tuberculosis who had not started taking antituberculosis drugs. Thirty four strains were isolated from patients taking antituberculosis drugs from more than 02 months.

Therefore this study shows both the primary as well as acquired drug resistance. In primary drug resistance 32.8% isolates were resistant to one or more drugs. The acquired drug resistance was very high, 67.6% isolates were resistant to one or more drugs.

INTRODUCTION

It is an increasing concern about the drug resistance of M. tuberculosis throughout the world especially in 3rd world countries.[1] Now the AIDS patients are not only susceptible to tuberculosis, but they can act as a source of spreading the disease (Tuberculosis) to healthy population.[2] The problem of tuberculosis is increasing day by day.[3] Tuberculosis is now leading cause of deaths in adults among the specific communicable diseases and will have killed at least 30 million people within next 10 years if current trends continue[3]. Now it is recommended that if it is not possible that each and every isolate of M. tuberculosis must be studied for sensitivity pattern but it is highly essential that after some time a study to find out the sensitivity pattern of M. tuberculosis in a particular area is done. This would help the physicians to choose the proper regimen of anti-tuberculosis drugs.[4]

MATERIALS AND METHODS

All the specimens were sputa. All the sputa were collected from clinically diagnosed cases of pulmonary tuberculosis. Total number of sputa collected was 217; out of 217 specimens, 72 were collected from admitted cases at Samli Sanatorium Murree taking antituberculosis drugs from more than 08 weeks, 145 specimens were collected from outdoor patients at Central Govt. T. B. Centre Rawalpindi. Out of 145 cases 127 were newly diagnosed cases, not yet started antituberculosis treatment, the rest 18 were taking antituberculosis drugs from more than 08 weeks.

All specimens were innoculated on two types of media, LJ with glycerol and LJ with pyruvate, after decontaminating with Modified Petroff's method.[5,6]

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One hundred isolates were finally selected for sensitivity testing, 2 isolates did not grow on control media, therefore they were excluded from study. Finally 98 isolates were studied for sensitivity testing. Resistance ratio method was used for sensitivity testing. Sensitive strain H37 Rv was used for comparison.[7,8]

LJ medium with glycerol was used for sensitivity purpose. Drugs were in corporated in LJ medium before inspissation[7].

Following drugs with these concentrations were used:
Streptomycin - 1,2,4,8,16,32,64mg/L.
INH - 0.025,0.05,0.1,0.2,0.4 & 1mg/dl
Ethambutol - 0.5,1,2,4mg/L
Rifampicin - 2,4,8,16,32,64 mg/L.[9,10]
Pyrazinamide: For pyrazinamide, 3 pairs of media were used, the pH for each media was 5.0,5.2 & 5.4. To one bottle 40.0mg/L was added, the other was kept without drug as a control.[11]

Reading was done after 04 weeks, twenty or more colonies were taken as positive growth. Resistance ratio of 08 was taken as resistant, resistance ratio 04 was repeated, if resistance ratio again came as 04, it was taken as resistant, less than 04 was taken as sensitive.[12,13]

RESULTS

Primary drug resistance to one or more drugs was 32.8% and two or more drugs was 15.62%.

Acquired drug resistance to one or more drugs was 67.64% & two or more drugs was 50% (Table-1).

TABLE-1: Resistance rate of 98 strains of M. tuberculosis to the commonly used drugs (Streptomycin, Isoniazid, Ethambutol, Rifampicin and Pyrazinamide).

Table - 1: Strains resistant to one or more drugs.

Primary	Acquired
32.81 % (21)	67.64 % (23)

Primary drug resistance to streptomycin was 9.37%, isoniazed 15.62%, rifampicin 7.81% and pyrazinamide 14.06% (Table-2).

TABLE - 2: Primary drug resistance of 64 strains of M. tuberculosis isolated from patients suffering from pulmonary tuberculosis.

Strains resistant to	
Streptomycin	0 9.37% (6)
Isonaizid	15.62% (10)
Ethambutol	04.68% (3)
Rifampicin	07.81% (5)
Pyrazinamide	14.06% (9)

Acquired drug resistance to streptomycin was 32.35%, isoniazed 41.17%, ethambutol 38.23%, rifampicin 38.23% and pyrazinamide 32.35% (Table-3).

DISCUSSION

Drug resistance of M. tuberculosis is a very important problem usually in under developed countries.[1] In this study both types of patients were studied, patient, who did not receive any antituberculosis drugs before and others who were taking antituberculosis drugs for several month. Patients who were taking anti- tuberculosis treatment showed a high degree of resistance, 67.6 % were resistant to one or more drugs. Most of these cases were hospitalized, this high degree of resistance may be associated with hospital. The strains which were resistant to 4 & 5 drugs all were isolated from admitted cases in the hospital.

TABLE - 3: Acquired drug resistance of 34 strains of M. tuberculosis.

Strains resistant to	
Streptomycin	32.35% (11)
Isoniazid	41.17% (14)
Ethambutol	38.23% (13)
Rifampicin	38.23% (13)
Pyrazinamide	32.35% (11)

No strain from out door patients showed resistance to more than three drugs.

Primary drug resistance is also quite high 32.81% isolates showed resistance to one or more drugs. Actual primary drug resistance might in slightly less than our study, because it happens that some of newly registered cases at T.B. Centre start taking antituberculosis treatment before registration at T.B. Centre. Our drug resistance appears to be higher as compared with developed countries, but they coincide with under developed countries.

In Australia primary drug resistance was 3.8%.[14] In Japan primary drug resistance was 8.5%.[15] In a study conducted at CDC in cooperation with 16 Public Health Laboratories, average primary resistance rate was 8.6%, but the rate varied markedly among various geographic areas; Asians and Hispanics had 20.7% & 15.0% respectively.[16]

In India average drug resistance to isoniazid was 14.7% and streptomycin 12.5%.[1] A study done by PMRC Islamabad showed that 32.4% isolates were resistant to one or more drugs. Resistance rate of streptomycin was 32.4%, isoniazid 28.3%, ethambutol 9.4% and rifampicin 10.8%. In our study average drug resistance against one drug (Pretreatment + during treatment) to streptomycin was 17.3%, isoniazid 24.4%, ethambutol 16.3% & rifampicin 18.36%.[17]

Average (primary + acquired) drug resistance to streptomycin was 17.3%, isoniazid 24.48%, ethambutol 16.32%, rifampicin 18.36% and pyrazinamide 20.4% (Table-4).

TABLE-4: Average (primary + acquired) drug resistance of 98 strains of *M. tuberculosis*.

Strains resistant to	
Streptomycin	17.34% (17)
Isoniazid	24.48% (24)
Ethambutol	06.32% (16)
Rifampicin	18.35% (18)
Pyrazinamide	20.40% (20)

Primary drug resistance to one drug was 17.18%, two drugs 10.93%, three drugs 4.68%, four & five drugs zero percent (Table-5).

Acquired drug resistance to one drug was 14.7% two drugs 11.76%, three drugs 20.58%, four drugs 8.82% and five drugs 8.82% (Table-6).

TABLE - 5: Primary drug resistant of 64 strains of M. tuberculosis according to number of drugs (streptomycin, isoniazid, ethambutol, rifampicin, pyrazinamide).

Sensitive to all drugs	67.18%(43)
Resistant to:	
One drug	17.18%(11)
two drugs	10.93%(7)
three drugs	4.68%(3)
four drugs	0.0%(Nil)
five drugs	0.0% (Nil)
TOTAL	100% (64)

TABLE - 6: Acquired drug resistance of 34 strains of M. tuberculosis according to number of drugs (streptomycin, isoniazid, ethambutol, rifampicin & pyrazinamide)

Sensitive to all drugs	35.29%(12)
Resistant to:	
one drug	14.7% (5)
two drugs	11.76%(4)
three drugs	20.58%(7)
four drugs	8.82% (3)
five drugs	8.82% (3)
TOTAL	100% (34)

Increase in drug resistance could be because of following reasons.

1. Improper regimens: physicians, surgeons, general practitioners and even quacks all are allowed to treat the tuberculosis patients. The drugs are freely available in the market. They can be purchased without prescription, selection of wrong regimens could increase drug resistance.

2. Intermittent treatment: Due to lack of education or cost of drugs, patients who are treated at private clinics have to buy drugs from the market cannot take treatment regularly. Even at Govt. Hospitals the drug supply is not regular, such an interrupted treatment can increase the rate of drug resistance.

3. Early cessation of drug regimens: Early cessation of drug is very frequently noted when the patients are relieved of symptoms or they cannot tolerate drugs they stop taking anti-tuberculosis treatment. Such an early cessation of drugs can lead to drug resistance.

SUGGESTIONS

- Treatment of tuberculosis should be restricted at T.B. Centres only.
- Antituberculosis drugs should not be allowed to be sold in the market.
- Drugs should be available at T.B. centres only.
- T.B. centre should be present at every district level so that they are within the reach of patients.
- Drug should be free of cost with regular supply.
- DOTS (Directly observed treatment short course) Strategy may be promoted as recommended by W.H.O local council, welfare societies and volunteers may be involved.
- Every T.B. centre should have a laboratory with a trained staff, where AFB staining and culture is done.
- Sensitivity testing should be done at reference laboratory.
- Every year a random study for sensitivity tests of M. tuberculosis should be done at different parts of the country.

Any patient who does not respond with initial few months' treatment should be asked for sensitivity testing at reference Centres. Results of such testing should be published regularly.

2nd line of drugs should be available at reference centres for resistant cases [18,19].

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