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Mycobacterium tuberculosis Susceptibility Testing Against Eight Anti-tuberculos Drugs in Lahore (Pakistan)

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Abstract

A total of 120 patients with positive cultures were tested with eight commonly used anti-tuberculosis drugs i.e. Isoniazid (H), streptomycin (S), Para aminosalicylic acid (PAS), Ethionarnide (ETH), Ethambutol (E), Thiacitazon (THI), Rifampicin and Pyrazinamide (Z). Standard proportion method as recommended by CDC Atlanta, Georgia (USA) using L-J medium was adopted. The isolates of M. tuberculosis were identified by their acid-fast character on Z-N staining, clonial growth on medium, niacin accumulation, nitrate reduction, positive catalase test and amplification of a 541-bp fragment from IS986 gene by Polymerase Chain Reaction (PCR). Sixty one (50.83%) of the isolates were resistant to one or more tuberculosis drugs. Of these resistant patients, 10 (16.39)%) were resistant to only one anti tuberculosis drug while (83.6%) were resistant to more than one drug. As regards resistance to individual drugs, 33 (27.5%) of the isolates were resistant to H, 22 (18.33%) to S, 42 (35%) to PAS, 21 (17:5%) to ETH, 20.0 (16.67%) to E, 8 (6.67%) to THI, 24 (20.0 to R and 34 (28.34%) to Z. These findings showed a growing concern of increasing prevalence of drug resistan tuberculosis.

Introduction

Tuberculosis (TB) is a highly contagious disease, caused by *Mycobacterium tuberculosis. M. tuberculosis* is the leading cause of death world wide from a single infectious disease (Centers for Disease Control, 1993). It is estimated that 1 billion individuals are infected world wide, with 8 to 10 million new cases and 2 to 3 million deaths per year (Kochi, 1991). The estimated cases of TB in Eastern Mediaterranean Region was 7.45 million (Dolin *et al*., 1993). In Pakistan 1.5 million suffer TB and more than 0.2 million new cases occurs yearly (Anonymous, 1997). The emergence of multi-drug resistant tuberculosis (MDR-TB) has led to a high mortality rates (Fischl *et al*., 1992). Drug resistance in TB is the decrease in sensitivity of tubercle bacilli to anti-tuberculosis drugs while it is reasonably certain that the concerned strain is not from the sample of wild strain which has never come to contact with drugs (WHO, 1991). Multiple drug resistance tuberculosis (MDR-TB) has emerged as a major public health concern in Pakistan in the last decade (Javaid, 1997). It seems that to date, Pakistan is losing the war against TB. Over halt the country has little or no access to health care. Approximately 1/4 (25%) of new TB cases are even diagnosed and only a fraction of doctors know how to prescribe effective treatment. Likely drug resistant strains are increasing at an alarming rate (Anonymous, 1997).

Prevalence of primary resistance to Rifampicin (H) has been reported to be 17 percent in Pakistan (Aziz *et al*., 1989), 7 percent in Kingdon of Saudi Arabia (Zaman, 1991) and 0.04 percent in United Kingdon (Medical Research Council, 1985). The initial drug resistance to other anti-tuberculosis drugs has varied from 5-30 percent all over the world (Anonymous, 1981).

In 1990 the first recognized clusters of primary resistance tuberculosis among hospitalized patients of AIDS in the United States were reported to the Centers Disease Control and Prevention (Centers for Disease Control, 1991). To date twelve such clusters have been reported There have been nosocomial outbreaks of TB caused organisms resistant to multi-drugs and reports of increased proportions of patients with drug resistant *Mycobacterium tuberculosis* at New York, City hospitals (Steiner *et al*., 1986).

Drug resistance complicates efforts to control tuberculos. Patients infected with organisms resistant to Rifampinin have a high rate of treatment failure when given the course (6-months) of therapy (Mitchison and Nunn, 1986). Patients infected with organisms resistant to both Isoniazid (H) and rifampicin (R) require at least 18 months of these (Goble, 1986). The effectiveness of preventive therapy patients infected with drug resistant *M. tuberculosis* organisms is unknown (Villarino *et al*., 1992). Strian MDR-TB, some of which are resistant to as many as some drugs, are deadly to both HIV positive and HIV negative individuals (Villarino *et al*., 1992).

This study was initiated to test *M. tuberculosis* susceptibility to commonly used anti-TB drugs in area. This study may provide some guidelines for anti drug therapy in TB patients.

Materials and Methods

Specimens: A total of 1779 specimens were received in Pakistan Medical Research Council TB Research Center, Mayo Hospital Lahore, Pakistan for AFB culture and the sensitivity from August 1997 to February 1988. The

specimens were sputum, pus, bronchial washings, urine, pleural fluid, lymph node and semen (Table 1).

Processing of the specimens: (1) The specimens were digested and decontaminated, as the sputum is always contaminated with a variety of numerous non-acid fast commensal bacteria from mouth and throat. The sputa were homogenized and then concentrated by modified Petroff's method before inoculation of centrifuged deposit for culture (Laidlaw, 1989). (2) Direct microscopy was done after fining me centrifuged deposit for acid-fast bacilli by Ziehl Neilsen staining method. (3) Each sputum was inoculated ontwo and extra pulmonary on three slopes of Lowenstein Jensen medium (L-J medium). (4) All the slopes were incubated at 37°C for 5-weeks, with weekly examination for the appearance of growth.

Identification of the Isolates: The isolates of *M. tuberculosis* were identified by their acid-fast character on Z-N staining, colonial characteristics on L-J medium, niacin accumulation, positive catalase test, nitrate reduction and amplification of a541-bp fragment from IS986 gene of insertion sequences by PCR.

Susceptibility testing of the Isolates: Eight commonly used anti-tuberculosis drugs i.e. Isoniazid (H),streptomycin(S), para-amino salicylic acid (PAS), ethionamide (ETH), etharrbutol (E), rifampicin (R), thiacitazone (THI) and pyrazinamide (Z) were used for drug susceptibility testing of *M. tuberculosis.* Standard proportion method as recommended by CDC Atlanta, Georgia (USA), using L-J medium was adopted. The concentration (pg/ml) of anti-tuberculosis drugs used were `H` 0.2, 'S' 4.0, 'PAS' 0.5, 20.0, `E' 2.0, 'THI' 2.0, 'R' 4.0 and 'Z' 100. For eacn drug two L-J slopes were inoculated one with 10G3 and the second with 10G5 dilution. For each specimen, four: control's without drugs were also used. All the slopeswere, incubated in an atmosphere of 5-10 percent CO2, at 37EC for 4-5 weeks. Then the slants were examined for the appearance of growth and the lowest concentration of antibiotic showing no more then 0-20 colonies were taken as MIC.

Results

A total of 1779 specimens were processed from August 1997 to February 1998, in which *M. tuberculosis* was isolated in 363 (20.4%). The drug susceptibility results of only 120 cases could be interpreted during the course of this study. These included 65 from sputum, 43 from pus, 4 from bronchial washings, 3 each from urine and pleural fluid, 1 each from lymph node and semen (Table 1).

A total of 61 (50.83%) of the isolates were found resistant to one or more antibiotics. Of these resistance cases, 10 (16.39%) were resistant to only one anti-tuberculosis drug, while 51 (83.6%) were found resistant to more than one drug. As regards resistance to individual drugs, 33 (27.5%) of the isolates were found resistant to isoniazid, 22 (18.33%) to Streptomycin, 42 (35%) to PAS, 21 (17.5%) to ETH, 20 (16.67%) to Ethambutol, 8 (6.67%) to THI, 24 (20.0%) to Rifampicin and 34 (28.34%) to Z (Table 2). Of the multi-drug resistance cases, 12 (19.67%) of the patients showed resistance Of 2 drugs, 13(21.31%) to 3 drugs, 7 (11.47%) to 4 drugs, 6 (9.83%) to 5 drug, 5 (8.19%) to 6 drug and 4 (6.56%) to each 7 and B drugs (Table 3). Five TB patients were resistant to HRZES, 3 to HRZE, 5 to HR 2 to HRE and 4 TO HT.

Table 1: Nature of specimens used for drug susceptibility testing (n = 120)

Source	No. of isolates	%age
Sputum	65	54.17
Pus	43	35.80
B. Washings	4	3.33
Urine	3	2.50
Pleural fluid	3	2.50
L. Node	1	0.83
Semen	1	0.83
Total	120	100.00

Table 2: Drug resistance in TB patients to individual drugs (n = 120)

Antibiotics	No. of isolates	%age
Isoniazid (H)	33	27.50
Streptomycin (S)	22	18.33
PAS	42	35.00
Ethionamide (ETH)	21	17.50
Ethambutol (E)	20	16.67
Thiacetazone (THI)	8	6.67
Rifampicin (R)	24	20.00
Pyrazinamide (Z)	34	28.34

Table 3: Prevalence of resistant Mycobacterium tuberculosis (n = 61)

No. of antibiotics	Resistant isolates	%age
One drug	10	16.38
Two drugs	12	19.67
Three drugs	13	21.31
Four drugs	7	11.47
Five	6	9.83
Six	5	8.19
Seven	4	6.56
Eight	4	6.56
Total	61	100.00

Discussion

Patients who take the prescribed drugs irregularly have an increased chance of developing drug resistant TB. Resistance to anti-tuberculosis drugs was noted soon after the drugs were first introduced (Youmans, 1946). Drug resistance has serious consequences for tuberculosis control in general. Multi-drug resistant patient may spread resistant strain primarily to their family members. Drug

resistance in M. tuberculosis is an emerging problem in Pakistan as well. Multi-drug resistant tuberculosis is readily transmitted among hospitalized patients with AIDS (Edlin *et al*., 1992). Transmission of multi drug resistance and the prolonged infectivity of patients due to infective chemotherapy (Valway *et al*., 1994).

In this study, 50.83 percent of the isolates were found resistant to one or more anti-tuberculosis drugs. Maximum resistance was observed to PAS (35%). It was followed by isoniazid (27.5%). Resistance to Thiacetazone was very low (6.67%). This is due to the fact that this drug is very rare in Pakistan and is not freely available in the market. An overall high drug resistance has been observed in the present study, but the situation is not so disappointing because the resistance to H, R, S, E and Z is still low. When these drugs are used together, they can control the resistant. Only 4 of the isolates were resistance to all the tested 8 drugs. For these patients, it is desirable that the second and third line drugs should be made available. The resistance results primarily from poorly managed programs, which lack the financial resources or do not implement the basic policies for control as recommended by WHO. Inappropriate control of treatment, reduced funding for fight against TB, neglected control procedures, inadequate training of health care workers, closure of facilities for managing TB patients, poverty, drug abuse, deterioration of the social conditions land of public health infrastructure, patients access to anti-TB drugs favoring inadequate regimens among other are the factors that have contributed to the development of MDR-TB in the world in general and Pakistan in particular.

This high level of resistance may become a very serious problem as it can lead to outbreak of multi-drug resistant tuberculosis. High drug resistance can be controlled and decreased by timely diagnosis and effective chemotherapy of TB patients. This objective can be achieved firstly by keeping check on non-compliance of doctors who prescribe inadequate and unreliable combination of drugs. Secondly the TB patients must be identified early and quickly to prevent *M. tuberculosis* to acquire resistance to anti-TB drugs. The most sensitivity, specific and rapid laboratory methods for the diagnosis such as PCR based diagnosis must be utilized and when the case is diagnosed, they must be treated under supervised and controlled guidance with full and appropriate regiments of anti-TB drugs.

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