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Drug Resistance Pattern in Multidrug Resistance Pulmonary Tuberculosis Patients

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ORIGINAL ARTICLE

Drug Resistance Pattern in Multidrug Resistance Pulmonary Tuberculosis Patients

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ABSTRACT

Objective: To evaluate the frequency of drug resistance profiles of multidrug resistant tuberculosis (MDR-TB) isolates of pulmonary tuberculosis patients, against both the first and the second line drugs.

Study Design: An observational study.

Place and Duration of Study: The multidrug resistant tuberculosis (MDR-TB) ward of Ojha Institute of Chest Diseases (OICD), Karachi, from 1996 to 2006.

Methodology: Culture proven MDR-TB cases (resistant to both isoniazid and Rifampicin) were retrospectively reviewed. Susceptibility testing was performed at the clinical laboratory of the Aga Khan University. Sensitivity against both first and second line anti-tuberculosis drugs was done. Susceptibility testing was performed using Agar proportion method on enriched middle brook 7H10 medium (BBL) for Rifampicin, Isoniazid, Streptomycin, Ethambutol, Ethionamide, Capreomycin and Ciprofloxacin. Pyrazinamide sensitivity was carried out using the BACTEC 7H12 medium. During the study period MTB H37Rv was used as control.

Results: Out of total 577 patients, all were resistant to both Rifampicin and Isoniazid (INH). 56.5% isolates were resistant to all five first line drugs. Resistances against other first line drugs was 76.60% for Pyrazinamide, 73% for Ethambutol and 68.11% for Streptomycin. Five hundred and ten (88%) cases were MDR plus resistant to one more first line drug. Forty (07%) isolates were MDR plus Quinolone-resistant. They were sensitive to Capreomycin but sensitivity against Amikacin and Kanamycin were not tested.

Conclusion: There were high resistance rates in MDR-TB to remaining first line and second line drugs. Continuous monitoring of drug resistance pattern especially of MDR isolates and treatment in specialized centers is a crucial need for future TB control in Pakistan.

Key words: *Mycobacterium tuberculosis. Resistance pattern. Multidrug resistance. Capreomycin.*

INTRODUCTION

Resistance against *Mycobacterium tuberculosis* (MTB) is important in the sense that it has an implication in the control of tuberculosis. The terms used to describe resistance to anti-tuberculosis drugs are resistance among new cases (or primary resistance) and resistance among previously treated patients.[1] The resistance among previously treated patients may be due to faulty treatment like prescription of inadequate treatment regimens, interrupted availability or poor quality of drugs, or incomplete treatment adherence[2] while subsequent transmission of these resistant organisms to others will lead to development of disease which is resistant from the beginning called primary resistance.[3]

Pakistan is ranked eighth in terms of global estimated burden of tuberculosis cases. Multi-Drug Resistant (MDR) tuberculosis among new cases and MDR among previously treated patients is 3.2% and 35% respectively.[4] There are small studies on resistance pattern from Pakistan.

The objective of this study was to retrospectively determine the resistance pattern against both first and second line drugs in MDR-TB patients during a decade at a specialized tuberculosis treatment centre.

METHODOLOGY

It is a retrospective observational study conducted at MDR ward of Ojha Institute of Chest Diseases (OICD).

The microbiological records of 577 culture proven cases of MDR pulmonary tuberculosis patients registered at OICD from 1996 to 2006 were reviewed. The patients were labeled as MDR- TB if their sputum culture and sensitivity report show resistance against both Rifampicin (R) and INH. All the study patients had a previous history of TB and use of anti-TB drugs so all the cases had secondary MDR-TB excluded. All those isolates sensitivity against either Rifampicin or INH were excluded. All patients were admitted initially followed by treatment in the clinic of the Institute.

Ethical Review Committee of Ojha Institute of Chest Diseases, Karachi, approved the study.

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All sputum specimens were processed at Microbiology Department, the Aga Khan University Hospital, Karachi. The sputum were digested and decontaminated using the N-acetyl-Lcysteine-NaOH method, as recommended by Centres for Disease Control and Prevention (CDC).[5,6] Specimens were concentrated using centrifugation (3000 x g) for 30 minutes and sediment was used for AFB microscopy and culture.

Sputum smears were prepared and screened by Auramine Rhodamine fluorochrome staining. The positive slides were then confirmed by Kinyoun modification of Ziehl Neelson stain. Isolation of *Mycobacterium tuberculosis* (MTB) was performed using standard methodology.[7]

The specimens were inoculated on both liquid and solid media i.e. MGIT and Lowenstein Jensen (LJ) medium. For LJ slant 0·1 ml of concentrated specimen were inoculated and incubated for 8 weeks. MGIT vials were inoculated with 0·5 ml of specimen and incubated at 37°C after supplementation of medium with OADC and PANTA. Growth from the positive LJ slant and MGIT vials were first stained with Kinyoun and *M. tuberculosis* was identified by BACTEC NAP TB differentiation test, growth in PNB containing media, nitrate reduction and niacin accumulation.[7]

Susceptibility testing was performed using Agar proportion method on enriched Middle Brook 7H10 medium (BBL) at the following concentrations; Rifampicin 1ug/ml and 5 ug/ml, Isoniazid 0·2 ug/ml and 1ug/ml, Streptomycin 2 ug/ml and 10 ug/ml, Ethambutol 5 ug/ml and 10 ug/ml, Ethionamide 5 ug/ml, Capreomycin 10 ug/ml, Cycloserine 30 ug/ml and Ciprofloxacin 2 ug/ml.[8] Pyrazinamide sensitivity was carried out using the BACTEC 7H12 medium pH 6·0 at 100 μg/ml. During the study period MTB H37Rv was used as control with each batch of susceptibility testing.

Only resistance to the higher concentration were used for analysis to ensure selection of high level resistance strains. Isoniazid resistance was defined as resistance to the higher concentrations (1 ug/ml) of the drug. Rifampicin resistance was defined as resistance to the higher concentrations (5 ug/ml) of the drug. Streptomycin resistance was defined as resistance to the higher concentrations (10 ug/ml) of the drug. Ethambutol resistance was defined as resistance to the higher concentrations (10 ug/ml) of the drug.

At the time of study, AKL was not checking sensitivities against Amikacin and Kanamycin and only tested four classes of second line TB drugs-Ethionamide, Capreomycin, Cycloserine and Ciprofloxacin/Ofloxacin. Therefore, all MDR TB isolates resistant to Ciprofloxacin and Capreomycin were taken as extensively drug resistant tuberculosis (XDR-TB).

MDR was defined as resistance to at least Isoniazid and Rifampicin. Five drug resistant MTB was resistant to all five first line drugs including INH, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin.

Extensively drug resistant tuberculosis (XDR-TB) was defined as resistance to any Quinolone and to one of the injectable second line drugs in addition to MDR.

The statistical package for social science SPSS (release 11.0.5) was used to analyze the data. Descriptive analysis was done for demographic, clinical and laboratory data. The results were presented as mean with standard deviation (SD) and numbers (percentages).

RESULTS

During the study period, a total of 577 MDR-TB patients were enrolled. There were 345 (59.7%) males. The mean age was 32.44±12.64 years. All the patients had received more than one course of anti-tuberculosis treatment (ATT) in the past. All the patients belonged to low socioeconomic status. Resistance to both INH and Rifampicin i.e. MDR tuberculosis was seen in all patients. Resistance against other first line drugs were 76.60% for Pyrazinamide, 73% for Ethambutol and 68.11% for Streptomycin. Resistant pattern against first and second line ATT is presented in Figures 1 and 2.

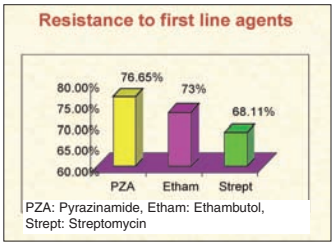

Figure 1: Resistance pattern of *Mycobacterium tuberculosis* against first line drugs.

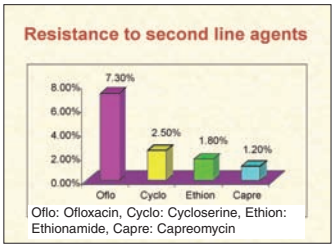

Figure 2: Resistance pattern of *Mycobacterium tuberculosis* against 2nd line drugs.

Three hundred twenty six (56.5%) were resistant to all five first line drugs, while 510 (88%) were MDR plus resistant to one more first line drug. Resistant pattern against different combinations of drugs are presented in Table I.

Forty (07%) isolates were MDR plus resistant to Quinolone. They were not tested against Amikacin or

Table I: Resistance pattern of MTB in MDR patients.

Antituberculous drugs	Resistance N (%)
MDR+ one more 1st line drug	510 (88%)
Resistant to all 5 first line drugs	326 (56.5%)
MDR + Quino	40 (7%)
Resistant to 5 first line drugs + Quino	06 (1.03%)
Resistance to different combinations of first line drugs	
RH only	62 (10.74%)
RHZ	34 (5.89%)
RHE	31 (5.37%)
RHEZ	36 (6.23%)
RHZS	09 (1.55%)
RHES	06 (1.03%)

MTB: Mycobacterium tuberculosis; MDR: Multidrug resistance; R: Rifampicin; H: Isoniazid; Z: Pyrazinamide; E: Ethambutol; S: Streptomycin; Quino: Quinolone.

Kanamycin, however, they were found to be sensitive to Capreomycin. Six (1.03%) isolates were resistant to all five first line drugs plus Quinolone.

DISCUSSION

This is the first large study on resistance pattern against first and second line anti-tuberculosis treatment (ATT) agents in MDR-TB patients. Previously published national and international studies were mainly focused on the primary and acquired drug resistance against first line antituberculous drugs in various group of patients. In this study, the focus was on the resistant pattern of first and second line ATT among MDR patients.

Very high resistance against all five first line drugs was noted as 56.5% in this study, which suggest faulty treatment in the past. In a global survey conducted by World Health Organization and International Union against tuberculosis and lung disease in 35 countries in five subcontinents resistance to 4 first line drugs (excluding Streptomycin) among previously treated patients was 0-17% with median of 4.4%.[9] The median prevalence of acquired MDR tuberculosis was 13%, with a range of 0% (Kenya) to 54% (Latvia).[9] Data from the recent survey from 66 countries and two Specially Administered Areas (SARs) of China in previously treated patients show highest proportions of MDR-TB in Baku City, Azerbaijan (55.8%) and Tashkent, Uzbekistan (60.0%) while data from India show 17.2%.[10]

Resistance to TB drugs has been widely reported from various parts of the country.[11-17] Study done by Irfan *et al.*[18] reported 35.2% of their MDR isolates were resistant to all five first line antituberculous agents. They showed an overall resistance rate of 64% to the antituberculous drugs. Other studies reported 36-73% resistance rates in different groups of patients.[11-17]

Karamat *et al.*, had reported single-drug resistance among *Mycobacterium tuberculosis* from both pulmonary and extra-pulmonary isolates at 21%, multi-drug resistance at 14% and resistance to all four first-line drugs at 5% in Rawalpindi-Islamabad.[14] The incidence of MDR was reported in local data from 21 to 24%.[11,15,17]

The very high resistance for all first line drugs is likely because only those cases were selected that were MDR and had a history of previous anti-TB treatment.

Resistance against second line drugs in this cohort of patients were low (1.2-2.5%) except for Quinolones (7.3%). So far, no data is available against the sensitivity of second line drugs in Pakistan, so these results remain unique. Even a low resistance rate of second line drugs is alarming for the treatment of this group of patients and will in lead to more XDR cases.

Forty (07%) isolates were MDR plus resistant to Quinolone with questionable XDR tuberculosis. The Quinolones are being used for large number of pulmonary indications like pneumonia, chronic bronchitis exacerbation, sinusitis etc. and over use of any single agent will ultimately result in resistance to the entire class.[19] Quinolone have been found very effective in the management of drug-resistant tuberculosis.[20]

All the patients had received multiple courses of anti-tuberculosis treatment (ATT) mostly from their general practitioners (GPs) and use of Quinolone is quite high among GPs of Pakistan. Most of the patients admitted that they would stop treatment at their own once they feel better. The detail treatment record was not available in almost all the patients. Studies have shown that there is a direct association between previous TB treatment and an increased prevalence of drug resistance.[18,21] To decrease resistance, adequate and complete therapy for patients diagnosed with tuberculosis should be ensured.

In Pakistan, 80% of the TB patients initially report to their general practitioners.[23] and involvement of GPs is crucial for effective TB control. They need to be sensitized and trained by organizing workshops, as most of them neither follow National Tuberculosis Control Program (NTP) guidelines nor practice Directly Observed Treatment Short course (DOTS). The problem of drug resistance can be overcome by DOTS strategy. It is said that effective TB control program leads to decrease in resistance pattern.[24] The Pakistan TB control program is working on achieving the WHO recommended target of 70% case detection and 85% treatment success rate. WHO's recent report (Ref-S) state that the case detection rate in Pakistan is increasing and is just below target at 67%, while the treatment success rate has reached 88%. The report correlates this increase with public-private mix (PPM) initiatives.

All the patients belonged to low socioeconomic status. Masroor and Ziaullah reported that low socioeconomic status is associated with increase number of drug resistant cases.[22,25]

There are certain limitations of this study; first, this study was done in a tertiary care referral TB centre so the very high resistance rate were expected and so these results cannot be generalized results for the whole community. Second, there were no result of second line drugs resistance in all cases so the resistance against second line drugs may be underestimated. Third, there is no data against Kanamycin and Amikacin so the prevalence of XDR in this cohort of cases could not be estimated.

CONCLUSION

High resistance rates in MDR-TB to remaining first line and second line drugs was found. Continuous monitoring and regular surveillance of drug resistance pattern especially of MDR isolates and treatment in specialized centres is a crucial need for future TB control in Pakistan.

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