Prevalence and pattern of Multidrug resistant tuberculosis among retreatment (Category II) patients of pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan

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AJ MZA ARK conceived idea, AJ MAK drafted the study, AJ MAK AG MZA collected data, AJ MAK did statistical analysis and interpretation of data, AJ MAK critical review manuscript, All approved final version to be published.

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The authors declare that there is no conflict of interest.

Abstract

Background: Resistance to the first-line drugs is a major issue in tuberculosis (TB) control. Globally 20% of previously treated TB cases have been reported to have multi-drug resistant tuberculosis (MDR-TB). Pakistan is among the top high TB burden countries with an annual incidence of 270 per 100,000 and estimated to have more than 13000 MDR-TB cases. In the province of Khyber Pakhtunkhwa (KPK) the annual incidence of TB is around 55,000 out of which around 5% are retreatment cases. So a survey was needed to determine prevalence of MDR-TB in KPK.

Objectives: Objective of the present study is to find out the prevalence of MDR-TB among previously treated (Category II) pulmonary tuberculosis patients in the province of Khyber Pakhtunkhwa, Pakistan.

Methodology: A total of 131 cultures positive retreatment patients were included in this cross sectional study. Drug susceptibility testing with Isoniazid, Rifampicin, Streptomycin, Ethambutol and Pyrazinamide were performed with standard technique.

Results: Of the 131 patients, 54 (41.2%) showed resistance to one or more drugs. Resistance with isoniazid and rifampicin [MRD-TB] was seen in 20 patients (15.3%). Resistance to streptomycin was highest (31.3%), followed by isoniazid (26.0%).

Conclusion: The result of this study showed prevalence of drug resistance of 41.2% and MDR-TB of 15.3% with the first line ATT among retreatment cases of pulmonary tuberculosis.

Keywords: Pulmonary Tuberculosis; Drug Resistance; Retreatment Patients; Pakistan

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Introduction

TB is leading infectious cause of death worldwide. Approximately one third of the world's population is infected with mycobacterium tuberculosis leading to 10 million new cases each year.[1-2] Adding to this the threat of emergence of multidrug-resistant tuberculosis (MDR-TB) poses a major risk against success of TB control programs. Worldwide emergence of multidrug resistance tuberculosis (MDR-TB) has been reported in both developed and the developing countries. Globally, 3.3% of new cases and 20% of previously treated cases are reported to have MDR-TB.[3]

The level of drug resistance is known to provide an epidemiological indicator to assess the extent of resistant bacterial transmission in the community as

well as success or otherwise of National Tuberculosis Programme (NTP). High levels of resistance have been reported in certain regions of the world particularly in Asia and parts of Africa.[4-10] The recommendation to use drug susceptibility tests for monitoring and guiding tuberculosis treatment programme was made many years ago.[11]

New TB patients are treated with successful first-line (Category I) regimen. Management of patients with a prior history of treated TB has been a cause of much concern.[12] For this group of patients, WHO recommends the use of retreatment regimen (category II) which has streptomycin added for first 2 months to four first line drugs and extended therapy for eight months. Treatment results of MDR-TB using this regimen tend to be relatively poor[13] and the effectiveness of Category II treatment for Category I failures has been doubtful especially in setting of a good DOTS, where most Category I failures have MDR-TB.[15-16]

WHO TB treatment guidelines recommended treatment guided by drug susceptibility testing (DST) for all previously treated patients.[13] In Pakistan, the guidelines recommend the standard category II regimen remain for all retreatment patients.

World Health Organization (WHO) however has recently been strongly recommending the use of Xpert MTB/RIF as the initial diagnostic test in adults and children suspected to have MDR-TB,[17] which simultaneously detects TB and rifampicin resistance (RR) in less than 2 hours. Whether or not this standard re-treatment regimen is appropriate depends on the prevalence of primary drug resistance among retreated cases. A study from Vietnam showed that 80% of patients who failed on category I treatment regimen were due to MDR-TB and cure rate with re-treatment regimen was 46%.[14] Another study from Uganda reported that the category II regimen for retreatment TB resulted in high failure rate.[18] A study from India reported 70% resistance among treatment failure and 49.3% among relapse patients[19] while a study from Multan, Pakistan reported high resistance pattern (60 to 75%) among category I failure patients.[20]

Pakistan ranks fifth TB high-burden country with an annual incidence of 270 per 100,000 and prevalence of 341 per 100,000 population.[3] A province wide prevalence survey of primary drug resistance reported 12.7% resistance to any of the first line drugs.[21] MDR-TB is becoming another major issue to compound the worsening condition and according to recent survey, based on 3.7% primary resistance and 18% resistance in re-treatment cases, WHO has estimated an annual incidence of about >13000 MDR-TB cases in Pakistan.[22]

In a province with 20 million population, where 5-10 % of all TB patients registered in DOTS are retreatment cases, a province wide resistance study was required to find out the prevalence of MDR-TB in patients with prior treatment.

Khyber Pakhtunkhwa is one of province of Pakistan. TB Control Programme, Khyber-Pakhtunkhwa is the only programme in the country, which has its own web based Electronic Recording and Reporting System. Programme Mission is TB free Khyber-Pakhtunkhwa. TB is a high priority agenda in the provincial Health Policy. An estimated 55,000 new TB cases occur in Khyber-Pakhtunkhwa every year.

The objective of this study was to assess the prevalence of MDR-TB in retreatment cases in the province Khyber Pakhtunkhwa (KPK), Pakistan.

Methodology

It was a cross sectional study to estimate the prevalence of drug resistance among retreatment TB patients in the province of Khyber Pakhtunkhwa. The study was approved by the Research and Ethics Committee of the Postgraduate Medical Institute, Lady Reading Hospital Peshawar.

The study was designed to determine resistance of Mycobacterium tuberculosis isolates from sputum cultures of retreatment smear positive TB patients. The Centres were located in the two main cities of KPK where patients from all over the province report. These included DOTS centres in Peshawar and Abbottabad. Out patient departments of Government Lady Reading Hospital Peshawar and Ayub Teaching Hospital Abbottabad and private clinics of all the investigators where patients from all over the province present themselves or are referred for consultation.

Table 1. Distribution of study subjects

Total No. of Subjects	153
Negative Culture	22 (14.4%)
Positive Cultures	131 (85.6%)
Susceptible to all 5 drugs	77 (58.8%)
Any Resistance	54 (41.2%

Table 2. Pattern of resistance to anti-tuberculosis drugs in study cases (n =131)

Drugs	n (%)
Total culture - positive	131 (100)
Fully sensitive to FLD	77 (58.8)
Any resistance to FLD	54 (41.2)
Resistance to the first line ATT	
Any Resistance	
SM	41 (31.3%)
INH	34 (26.0%)
PZA	26 (19.9%)
EMB	26 (19.9%)
RMP	21 (16.0%)
Mono Resistance	
INH	05 (3.8%)
RMP	00 (%)
EMB	03 (2.3%)
SM	15 (11.5%)
MDR	
INH + RMP	20 (15.3%)
Additional Resistance in MDR Strains	
Three drugs Resistance	
INH + RMP + PZA	1 (5.0%)
Four drugs Resistance	
INH + RMP + PZA + EMB	01 (5.0%)
INH + RMP + EMB + SM	01 (5.0%)
INH + RMP + PZA + SM	01 (5.0%)
Five drugs Resistance	
INH+RMP+SM+EMB+PZA	16 (80.0%)

INH isoniazid; RMP rifampicin; EMB ethambutol; SM streptomycin; PZA pyrazinamide

Patients Sputum smear examination was performed at the respective diagnostic centres where the patients suspected to have TB were screened. The retreatment patients fulfilling inclusion criteria with smear positive specimens were enrolled in the study and their sputum were sent to the collection centre of the central laboratory for culture and sensitivity testing. The sample size was calculated according to the population and WHO's estimated incidence of smear positive tuberculosis in the province/country. According to these calculations, a sample size of at least 122 was required.

The following inclusion criteria were used in the study:

- Smear-positive retreatment pulmonary TB patients
- Any sex and age
- Pakistani residents
- Previous history of anti-tuberculosis medication (treatment failure and relapse patients)

The Department of Microbiology Laboratory at the Aga Khan University Hospital was used as the central laboratory for culture and DST. The Aga Khan University TB laboratory is a WHO designated Supra National Laboratory (SRL) and participates in SRL network DST proficiency testing.

Processing of Sputum Specimens

Microbiological methods

Specimen processing

All samples were decontaminated with N-acetyl-L-cysteine (NALC) sodium hydroxide according to the standard protocol. All specimens were concentrated

by centrifugation for 30 min and sediments were used for acid-fast bacilli microscopy and culture.

Microscopy

Smears for microscopy were screened using auramine staining. Positive slides were further confirmed by staining with Kinyounmodification of Ziehl-Neelsen stain.

Isolation of M. tuberculosis

Mycobacterial cultures were performed on both liquid and solid media. Sediments were cultured at 37°C using Lowenstein-Jensen (LJ) medium and the Mycobacteria Growth Indicator Tube (Becton Dickinson Diagnostic Instruments Systems, Sparks, MD, USA). For the LJ slant, 0.1 ml of concentrated specimen was inoculated and incubated for 8 weeks. MGIT vials were inoculated with 0.5 ml of specimen and incubated at 37°C after supplementation of the medium with OADC (oleic acid-albumin-dextrose-catalase) and PANTA (Polymyxin B, Amphotericin B, Nalidixic Acid, Trimetho-Prim and Azlocillin). Growth from the positive LJ slant and MGIT vials were first stained with Kinyoun, M. tuberculosis was then.

Drug susceptibility testing (DST)

DST was performed using the standard agar proportion method on enriched Middlebrook 7H10 medium (BBL, BecktonDickinson) at the following drug concentrations: rifampicin(RMP) 1µg/ml, isoniazid (INH) 0.2 and 1µg/ml, streptomycin (SM) 2 µg and 10 µg/ml and ethambutol (EMB) 5 and 10 µg/ml [18,19]. In accordance with WHO recommendations the following concentrations were reported; rifampicin 1.0µg/ml, isoniazid 0.2 µg/ml, streptomycin 2.0 µg/ml and ethambutol 5.0 µg/ml Pyrazinamide (PZA) sensitivity was carried out using BACTEC 7H12 medium pH6.0 at 100 g/ml (BACTEC PZA test medium, Becton Dickinson, Sparks, MD, USA) in accordance with the manufacturer's instructions. M. tuberculosisH37Rv was used as a control with each batch of DST.

Statistical Analysis

The data was analyzed using SPSS v.16. Descriptive statistics were used to describe resistance pattern of

Table 3. Pattern of resistance to Second line anti-tuberculosis drugs in study cases (n =131)

Drugs	n (%)
Total culture - positive	131 (100)
Fully sensitive to SLD	90 (68.7%)
Any resistance to SLD	41 (31.3)
Resistance to the Second line ATT	
Any Resistance	
AK	01 (0.8%)
CAP	03 (2.3%)
ETH	03 (2.3%)
OFX	28 (21.4%)
PAS	07 (5.3%)
Mono Resistance	
CAP	03 (2.3%)
ETH	01 (0.8%)
OFX	23 (17.60%)
PAS	03 (2.3%)
XDR	
INH + RMP + AK + ETH + OFX + PAS	01 (0.8%)
Additional Resistance in MDR Strains	
INH + RMP + OFX	28 (21.4%)
INH + RMP + CAP	03 (2.3%)
INH + RMP + ETH	03 (2.3%)
INH + RMP + PAS	07 (5.3%)

OFX Ofloxacin; CAP capreomycin; ETH ethionamide; PAS Para-Aminosilicyclic Acid

individual drugs and their different combinations. Comparison of ATT resistance between treatment failure and relapse group was done by chi square test. P value of <0.05 was taken as significant.

Results

A total of 153 sputum samples were sent from the enrolment sites out of which 22 (14.4%) specimens were found to be smear/culture negative at the reference laboratory and hence excluded from the study and 131 (85.6%) found to be culture-positive at the central laboratory (Table1). Of the 131 patients, isolates from 77 (58.8%) were fully susceptible to all the first-line drugs tested, while 54 (41.2%) showed resistance to one or more drugs.

Of the 131 culture-positive patients, 63 (54.7%) were males and 68 (45.3%) females, with a mean age of 33.4 ±17.04 years.

The pattern of resistance to first line anti-tuberculosis drugs is tabulated in Table 2. Mono drug-resistance was observed in 23 (17.6%) patients. Single drug resistance was most commonly seen with Streptomycin (SM) (n=15, 11.5%), Isoniazid (INH) (n = 5, 3.8%) and Ethambutol (n = 3, 2.6%).

Resistance to streptomycin (SM) alone or in any combination was highest, observed in 41 (31.3%), followed by isoniazid 34 (26.0%), Pyrazinamide 26 (19.9%), ethambutol 26 (19.9%) and by rifampicin 21 (16.0%). Resistance with isoniazid plus rifampicin with or without any other drug [MRD-TB] was seen in 20 (15.3%) patients.

MDR cases with additional resistance to PZA was 1 (5.0%) among study cases. Among four drugs resistance (MDR plus two other drugs), 01 (5.0%) case were resistant to INH + RMP + PZA + EMB, INH + RMP + EMB + SM and INH + RMP + PZA + SM (Table 2 & 3).

Discussion

In the present study, we present the drug resistance profiles of re-treatment TB cases in the province of KPK Pakistan. In this study resistance to any 1st line drug was 41.2%, out of which 15.3% were MDR-TB. Most of the rifampicin resistant cases (95.2%) were also resistant to isoniazid. This has also been observed in other studies.[19-23]

A previous study of primary drug resistance from Pakistan showed that 11.3% were resistant to one or more of the first-line drugs while 1.8% had MDR-TB[24] as compared to 41.2% and 15.3% respectively in our study. This clearly provides evidence of much higher acquired drug resistance in Pakistan. The current situation may be attributed to factors like unknown drug quality, variability of drugs availability especially in public sector hospitals, inadequate regimens, lack of follow up, poverty, malnutrition, immune compromised states and lack of political commitment.

The present study showed that the single-drug resistance to streptomycin was the highest 31.3%. The problem is lower than those reported by other countries – 12.1% in South Africa, and 16% in Saudi Arabia.[25-26] Possible reason for such high rate is that SM is widely used in the treatment of other bacterial infections also and due to poor compliance by patient in their past TB treatment. Resistance to INH is 26.0%. Streptomycin and INH resistance must be seriously considered since these drugs are core components of the standard and short-course chemotherapy regimens. These are relatively cheap drugs with a vital role in the treatment of tuberculosis in developing countries. Losing the effectiveness of these drugs may mean changing the treatment regimen to a more expensive one, and even the current standard regimen, which is considered to be relatively cheap, is unaffordable for many countries in the developing world.

One alarming finding of the present study is additional three drugs resistance (i.e. SM+EMB+PZA) with MDR-TB (i.e. INH+RMP+SM+EMB+PZA) was 80%.

This study emphasizes the need for initial DST before starting treatment of re-treatment TB cases. Worldwide, there is now large data available which emphasize the need for DST to rule out MDR-TB before starting the treatment of retreatment TB cases. A study from Peru reported that approximately 75% of category-I failure patients had MDR-TB. Another study from Siberian prison reported 35% of treatment failure rate of category II.[27] Studies from Peru and Iran showed that treatment success of category-I failure according to category-II regimen only was quite low as compared to individualized management after DST.[28,29-16,17] Another study from Vietnam reported that 80% of category-I failure had MDR-TB as compared to only 8% relapse patients.[14]

It is recommended that NTP should document and use their country drug resistance data on relapse and failure cases to find out the levels of MDR-TB. This will provide an insight into the success of these programs as well as the extent of transmission of drug resistant in population.

This maiden study on retreatment cases in KPK, using standard method, gives reliable and valid baseline data which will be helpful for recommendation for evidence based treatment strategy in this group of patients.

Conclusion

This study has demonstrated that there is very high burden of drug resistance in treatment failure and

relapse patients of pulmonary TB. Finding drug resistance patterns and treatment with selective first line drugs and second-line anti-tuberculosis drugs is appropriate for these patients in order to reduce the emergence of multidrug-resistant tuberculosis in Pakistan.

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