ORIGINAL ARTICLE:

Primary Drug Resistance to Antituberculous drugs in Punjab Pakistan.

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ABSTRACT:

Objective: To assess the prevalence of Primary drug resistance in Punjab

Introduction/ Methods: Tuberculosis is a serious public health problem. Pakistan ranks 6th in terms of TB burden with a WHO estimated incidence rate of 181 per 100,000 persons. This study was a cross-sectional prevalence study, evaluating the prevalence of drug resistance among new TB patients, using a non-probability convenience sampling methodology. The sample size was calculated according to the population & WHO's estimated incidence of smear positive tuberculosis in the province/country. Sputum samples were obtained from 430 newly diagnosed patients of pulmonary tuberculosis from various centres in Lahore, Rawalpindi & Multan.

Results: Sensitivities were performed by proportion method which showed the following resistance values in 387 eligible patients. 42 (**10.8 %**) samples showed primary resistance to one or more drugs. 28 (**7.2%**) of the isolates tested were resistant to a single drug, 8 (**2.0%**) were resistant to 2 drugs, 2 (**0.5%**) to 3 drugs, 3 (**0.75%**) to 4 drugs while one (**0.25%**) to all 5 first line agents.

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Resistance to Streptomycin (10µg/ml) was seen in 19 **(5.9%)**, Isoniazid (1µg/ml) in 27 **(7.0%)**, Rifampicin (5µg/ml) in 5 **(1.2%)**, Ethambutol (10µg/ml) in 9 **(2.3%)** and Pyrazinamide in 7 **(1.8%)** samples. Primary Multidrug resistance was in 4 (**1.0** %) patients (Isoniazid 1µg/ml, rifampicin 5 µg/ml with or without other drugs).

Conclusion: This study from province wide samples suggests that prevalence of MDR amongst untreated patients in Punjab is **1.0%,** which is a cause of concern and should be addressed through effective TB control programs with DOTS strategy.

Keywords:
Primary drug resistance, Pulmonary Tuberculosis (PTB), Mycobacterium Tuberculosis (MTB), MDR-TB, Punjab.

INTRODUCTION:

Tuberculosis is a serious public health problem in the developing countries. Worldwide emergence of MDRTB has been reported in both developed and the developing countries and poses a major threat to the control of TB.[1-2] Drug resistance is primary when it develops in a person who has not been exposed to anti TB treatment in the past. This is in contrast to acquired drug resistance, which is present in previously treated patients with inadequate or irregular chemotherapy. World Health Organization-International Union against Tuberculosis and Lung Disease from a global surveillance for antituberculosis-drug resistance, reported the prevalence of Primary MDR at 1.4% and 13% in previously treated patients[3].A survey from 48 geographic sites revealed that drug resistant tuberculosis is ubiquitous and median prevalence of primary resistance to at least one drug in around 10.7 % and that of Primary MDR only 1%.[4]

In Pakistan, the incidence of TB is estimated at 181 per 1, 00,000 population and each year at least 286,000 new TB cases are added to the existent patient population of around 1.8million[5]. 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 & parts of Africa.[6-12] The recommendation to use drug susceptibility tests for monitoring and guiding tuberculosis treatment programme was made many years ago.[13] In view of the practical difficulties in collecting comparable data, the World Health Organization (WHO) proposed a programme of global surveillance of drug resistance in Tuberculosis through its collaborating centres for bacteriology of tuberculosis which would function as supranational Reference Laboratories (SRL) for the respective regions. The proposed programme was based on random sampling of patients reporting to clinics for tuberculosis treatment. Susceptibility testing was to be performed by the reference Laboratories based on a common protocol including uniform Laboratory methods. As a first step, a regional survey was carried out in 10 Latin American countries[14]**.** The overall experience gained in Latin America suggested that a sample survey of drug resistance with large failure rates of more than 5% may indicate inadequate routine treatment and high levels of initial resistance, which made survey of drug resistance a priority.[14]

Several countries in Asia and Africa undertook national surveys in accordance with the protocol. Countries including Tanzania[15]**,** South Africa[16] and India[17] established systematic national surveillance programmes. In Pakistan no such national level survey has ever been carried out. In a country ranked 6th in the world in terms of TB disease burden and with 45% of TB disease burden of EMRO region, a country wide resistance

surveillance study was badly needed in order to determine the prevalence, pattern and trends of anti TB drug resistance in the country**.**

The fact that DOTS strategy has not been implemented in the country until recently and TB patients by and large have been treated unsupervised, it was assumed that primary drug resistance is likely to be high in Pakistan. This hypothesis was supported by reports from different cities of Pakistan pointing towards a high drug resistance in the country, one study showed resistance to Rifampicin ((R)) and Isoniazid (INH) to 15% and 11 % respectively[20] and in another one resistance to H 25%, R 15%, E 12% and S 12%[21]. A study from NWFP in 1994 also showed relatively high primary and acquired drug resistance[24].

In order to test this hypothesis a study was conducted on patients from all over the province presenting to diagnostic centers in the main cities of Punjab i.e. Lahore, Multan & Rawalpindi. The objective of this study was to assess the prevalence of primary drug resistance in the province.

METHODS:

The study was designed to determine resistance of Mycobacterium tuberculosis isolates from sputum cultures of newly diagnosed smear positive TB patients, which were collected from patients of Punjab Province presenting with features of TB to diagnostic centers in Lahore, Multan & Rawalpindi.

The centers where all the 7 investigators worked in different parts of the province formed the diagnostic centers. These Centers were located in the three main cities of Punjab which has the catchment population of patients from all over the province. These included TB control Programme centers, out patient departments of Mayo, Gulab Devi, Nishtar hospitals and private clinics of all the investigators where patients from all over the province present themselves or are referred for consultation. In all a total of 12 centers participated in the study.

Subjects Sputum smear examination was performed at the respective diagnostic centers where the patients suspected to have TB were screened. The subjects with smear positive specimens were enrolled in the study and their sputum were sent to the collection centre of the central laboratory for culture & sensitivity testing provided the patient has not used anti TB treatment in the past and fulfilled all the inclusion criteria.

This study was basically a cross-sectional prevalence study, using a non-probability convenience sampling methodology. The sample size was calculated according to the population & WHO's estimated incidence of smear positive tuberculosis in the province/country. According to these calculations, a sample size of at least 422 was needed for the study which was collected over a period of 6 months. A sputum specimen in the container provided by the central laboratory along with a form was filled

by the investigator at the diagnostic centre, giving beside other details a declaration by the investigator that he has confirmed that the patient has never taken anti-TB drugs in the past. Patients were given an information leaflet and informed consent was taken from all patients.

Inclusion Criteria:

New Smear Positive Pulmonary T.B Patients

Any Sex & Age

Resident of Punjab

No prior anti-T.B medication. Naïve patients.

Sputum collected for Culture before start of treatment.

The Department of Microbiology laboratory at Aga Khan University Hospital was used as the Central Laboratory for culture & sensitivity testing.

Smears for microscopy were screened using Auramine Rhodamine staining. Positive slides were further confirmed by staining with Kinyoun modification of Ziehl Neelson stain.

Mycobacterial cultures were performed on both liquid as well as solid media. Sediments were cultured at 37^{o}C using Lowenstein Jensen (LJ) medium and MIGIT (Becton Dickinson Diagnostic Instruments Systems). For 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^{o}C after supplementation of medium with OADC and PANTA; containing Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocilin. 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 (Becton Dickinson, USA).

Susceptibility testing was performed using standard agar proportion method on enriched Middle brook 7H10 medium (BBL) at the following final drug concentrations; rifampicin 5ug/ml, isoniazid 1ug/ml, streptomycin10ug/ml and ethambutol10ug/ml [18,19]. Disc elusion sensitivity plates were prepared using paper sensitivity disc (BBL). McFarland No. 1 standard suspension of isolate was made from growth on LJ slant and diluted to 10^{-2} and 10^{-4} dilutions. The inoculated plates were incubated at 35^{o}C and examined for growth each week for 8 weeks. *M.tuberculosis* was considered resistant to a given drug when growth ≥1% above the antibiotic free control was observed in drug containing area. Pyrazinamide sensitivity was carried out g/ml (BACTEC using the BACTEC 7H12 medium pH6.0 at 100 ^{TM} PZA test medium, Becton Dickinson USA) in accordance with manufacturers instructions. MTB H37Rv was used as control with each batch of susceptibility testing.

Data Analysis

Data was analyzed using SPSS version 10. The results are presented in the form of Tables and graphs.

RESULTS:

430 samples were evaluated out of which 388 (90%) samples were found to be culture positive.

On smear examination 26 (6.7%) of 388 smear positive specimens collected from diagnostic centres from across the province were found to be smear negative at the reference laboratory.

Drug susceptibility results were positive on 388. One sample grew Mycobacterium other than Tuberculosis (MOTT) and hence was excluded from further analysis. Out of the remaining 387 patients, the isolates from 345 (89.1%) patients were fully susceptible to all the 1st line drugs tested, while 42 (10.8%) patients showed resistance to one or more

drugs. Resistance to Isoniazid alone or in combination with other drugs was seen in 27 (7.0%) patients. Similarly resistance to streptomycin, Rifampicin, Ethambutal & Pyrazinamide was seen in 19(5.0%), 5(1.2%), 9(2.3) & 7(1.8) respectively. (Table 1)

Resistance to 1 drug was seen in 28 (6.8 %) patients, 2 drugs in 8 (2.0%) patients, 3 drugs in 2 (0.5%) patients, 4 drugs in 3 (0.75 %) patients & one (0.25%) sample showed resistance to all 5 drugs. Primary MDR was found in 4 (1.0 %) patients. (Table2).

DISCUSSION:

The WHO/IUATLD Global Project on Anti- tuberculosis Drug Resistance Surveillance recorded considerable variation in the prevalence of drug resistance among 35 countries in 5 continents. The median prevalence of Primary drug resistance was 9.9% with range of 2% to 41%. Overall, the median prevalence of primary MDR-TB was 1.4% ranging from 0 to 10% .Among the South East Asian Region (SEAR) countries, the prevalence of primary resistance is readily available only for Nepal and Thailand since they participated in the WHO supported Global Project on Anti- tuberculosis Drug Resistance Surveillance in 1994-97. The median prevalence of acquired resistance to any drug was recorded as 23.2% with range of 9.8% to 36.6 %. The median prevalence of primary MDR-TB was 2.5 % significantly higher than the global mean of 1.4 %[3]. However such information for other (SEAR) countries, based on standardized protocols and methods, is not available.

Although drugs resistance tuberculosis has frequently been encountered in Pakistan and its presence has been known, there was no comprehensive report mainly due to limited facilities available for culture and susceptibility tests across the country. The present study on drug resistance in Pakistan using internationally acceptable guidelines and a standardized methodology gives reliable baseline information.

This study reveals culture positively of 90% of all the smear positive patients, confirming the quality of reference laboratory at AKU which was also up to the acceptable standard. The level of drug resistance to Isoniazid, Rifampicin, and MDR of 7.0%, 1.2 %, and 1.0 % respectively in previously untreated cases, as is evident from this study, is not as high as one would have expected, keeping in view that Punjab has recently reached the WHO target of 100% DOTS coverage. These values are comparable with resistance studies in different 3rd world countries. However the result of this study is different from other surveys conducted in Pakistan. According to a study by Khan JA, et al, the primary resistance to Isoniazed and Rifampicin was found to be 11% and 15% respectively [20]. In another study resistance was found to be even higher with H 25%, R 15%, E 12% and S19%.[21] This relatively high percentage of resistance to individual drugs in the studies from Pakistan[22] was perhaps due to the faulty selection of patients and it appears that efforts were not made to separate primary drug resistance from initial or acquired resistance. In view of the above mentioned studies, it was realized that there is a strong need to evaluate the primary resistance to Anti-tuberculosis drugs in various provinces and the whole country.

There has perhaps been a gradual increase in primary drug resistance over the years. This could be overcome by a strong control programme with DOTS strategy which can reduce the emergence of drug resistance in the community. Since no newer drugs for tuberculosis are likely to become available in the near future, the only options left for the prevention of drugs resistance are effective case finding, prompt and correct diagnosis and successful treatment of patients. Apart from a strong control programme, continuous surveillance of drug resistance will provide information which will serve as a useful parameter in the evaluation of control programs.

CONCLUSION:

A poorly functioning program can create MDR-TB much faster than it can be treated, even if unlimited resources are available. There is no single prescription for controlling MDR-TB but the various tools available should be applied wisely**.** Adoption of DOTS to prevent the generation of resistant strains and careful introduction of second-line drugs to treat patients with MDR are the top priorities for proper control/containment of MDR-TB.

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Contributors

All authors participated in the data analysis and development of manuscript, and saw and approved the final version.

Conflict of interest statement

We all the authors declare that we have no conflict of interests.

Role of funding source

The study was financed by the Wyeth Pharmaceutical (Pvt) Ltd. The sponsor had no role in data collection, data analysis, data interpretation or writing of the report. The corresponding author had access to all data in the study and had final responsibility for the decision to submit for publication.

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Table: 1 Summary of Susceptibility Reports- 387 samples

RESISTANT TO:	FREQUENCY	PERCENT
Streptomycin 10µg	**19**	**5.0 %**
Isoniazid 1µg	**27**	**7.0 %**
Rifampicin 5µg	**05**	**1.2 %**
Ethambutol 10g	**09**	**2.3 %**
Pyrazinamide 100 g	**07**	**1.8 %**
MDR	**04**	**1.0 %**

Table 2: ***RESISTANCE PATTERN OF MYCOBACTERIUM TB (42 RESISTANT SAMPLES)***

	Numbers	**% age**
Total Culture +ve	387	100.0
Fully sensitive	345	89.1%
Any Resistance	42	10.8%
Resistance to		
Only H	13	3.3%
Only R	01	0.25%
Only E	03	0.75%
Only S	04	1.0%
Only P	0	0%
HE	04	1.0%
HR	0	0%
HP	01	0.25%
HS	03	0.75%
HSP	02	0.50%
HEP	0	0%
HRP	0	0%
HRSP	02	0.50%
HREP	01	0.25%
HRSEP	01	0.25%
Any H Resistance	**27**	**7.0%**
Any R Resistance	**05**	**1.2%**
Any HR Resistance	**04**	**1.0%**

Key

H= Isoniazid
R=Rifampicin
E=Ethambutol
S=Streptomycin
P=Pyrazinamide