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Primary Drug Resistance to Anti-tuberculous Drugs in Karachi

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ABSTRACT

Objective: To assess the frequency of primary drug resistance among newly diagnosed tuberculosis cases in Karachi.
Study Design: Cross-sectional study.
Place and Duration of Study: Multicentric study involving various TB clinics and treatment centres of Karachi between April to December 2005.
Methodology: The frequency of drug resistance among new TB patients was evaluated using a non-probability convenient sampling methodology. Sputum sample was obtained from 140 newly diagnosed sputum smear-positive patients of pulmonary tuberculosis from various centres of Karachi. Sensitivities were performed by proportion method.
Results: Fifteen (11.5%) samples in 130 eligible patients showed primary resistance to one or more drugs. Ten (7.6%) of the isolates tested were resistant to a single drug, none were resistant to 2 drugs, 4 (3.0%) to 3 drugs and 1 (0.76%) to 4 drugs while 1 (0.76%) to all 5 first line agents. Resistance to streptomycin (10 ug/ml) was seen in 8 (6.1%), isoniazid (1ug/ml) in 12 (9.2%), Rifampicin (5 ug/ml) in 4 (3.0%), ethambutol (10 ug/ml) in 1 (0.76%) and pyrazinamide in 6 (4.6%) samples. Primary Multi-Drug Resistance (PMDR) was found in 2 (1.5%) patients. (Isoniazid 1 ug/ml, rifampicin 5 ug/ml with or without other drugs).
Conclusion: In the studied patients, primary drug resistance to at least one anti-tuberculosis drug was 11.5%. It requires an efficiently working anti-tuberculosis programme to prevent escalation including resistance.

Key words: *Primary drug resistance.   Pulmonary tuberculosis (PTB).   Mycobacterium tuberculosis (MTB).*

INTRODUCTION

Worldwide emergence of multi-drug-resistant tuberculosis (MDR TB) has been reported in both developed and the developing countries and poses a major threat to the control of TB.[1,2] MDR TB, defined as resistance to at least isoniazid and rifampicin poses a major threat to tuberculosis control programme, since the treatment of such cases is complex, more expensive and frequently less successful than treatment of non-resistant strains.[3] Primary drug resistance is defined as resistance that develops in a person who has not taken anti-TB treatment in the past. This is opposed to acquired drug resistance, which is present in previously treated patient with inadequate or irregular chemotherapy. World Health Organization International Union against tuberculosis and lung disease reported

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the prevalence of Primary MDR at 1.4% and 13% in previously treated patients from a global surveillance for anti-tuberculosis drug resistance.[4] A survey from 48 geographic sites revealed that drug resistant tuberculosis is ubiquitous and median prevalence of primary resistance to at least one drug is around 10.7% and that of Primary MDR only 1%.[5]

In Pakistan, the incidence of TB is estimated at 181/100,000 population and each year at least 286,000 new TB cases are added to the existent patient population of around 1.8 million.[6] 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.[7-13] Several countries in Asia and Africa undertook national surveys to see the prevalence of drug resistance. Countries including Tanzania, South Africa and India established systematic national surveillance programmes.[14-16] In Pakistan, no such 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, resistance surveillance studies are required to determine the frequency, prevalence, pattern and trends of anti-TB drug resistance in the country.

The DOTS strategy has only been recently implemented in the country 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[17] and in another one, resistance to H 25%, R 15%, ethambutol (E) 12% and S 12%.[18] A study from NWFP in 1994 also showed relatively high primary and acquired drug resistance.[19]

The aim of this study was to determine the frequency of primary anti-tuberculous drug resistance in the city of Karachi.

METHODOLOGY

The study was approved by Research and Ethics Committee of Postgraduate Medical Institute, Peshawar, Pakistan. 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 Karachi presenting with features of TB to diagnostic centres from April to December 2005.

The centres where all the 4 investigators worked in different parts of the city formed the diagnostic centres. These included TB control programme centres, out-patient departments of JPMC, Liaqat National Hospital, Ojah Institute of Chest Diseases, Lyari General Hospital and private clinics of all the investigators.

Subjects' sputum smear examination was performed at the respective diagnostic centres, 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 and sensitivity testing provided the patient has not used anti-TB treatment in the past and fulfilled all the inclusion criteria.

The sample size was calculated according to the population and World Health Organization's (WHO) estimated incidence of smear-positive tuberculosis in the country. Accordingly, a sample size of at least 140 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 were new smear-positive pulmonary TB patients, resident of Karachi, with no prior anti-TB medication, whose sputum were collected for culture before start of treatment. Patients who had used some form of anti-TB treatment in the past were excluded.

The Department of Microbiology Laboratory at Aga Khan University Hospital was used as the central laboratory for culture and sensitivity testing. Smears for microscopy were screened using Auramine Rhodamine staining. Positive slides were further confirmed by staining with kinyoun modification of Ziehl Nelson stain.

Mycobacterial cultures were performed on both liquid as well as solid media. Sediments were cultured at 37⁰C using Lowenstein Jensen (LJ) medium and MIGIT. 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 37⁰C 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.

Susceptibility testing was performed using standard agar proportion method on enriched Middle Brook 7H 10 medium at the following final drug concentration; rifampicin 5 ug/ml, isoniazid 1 ug/ml, streptomycin 10 ug/ml and ethambutol 10 ug/ml.[20,21] Disc elusion sensitivity plates were prepared using paper sensitivity disc. 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 35⁰C and examined for growth $\geq 1\%$ above the antibiotic free control was observed in drug containing area. Pyrazinamide sensitivity was carried out 100 g/ml (BACTEC using the BACTEC 7H 12 medium pH 6.0 at 100™ PZA test medium in accordance with susceptibililty testing.

Data was analyzed using SPSS version 10.

RESULTS

One hundred and forty samples collected from eight centres were evaluated, out of which 131 (93.5%) samples were found to be culture-positive.

On smear examination, 7 (5.3%) of 131 smear-positive specimens collected from diagnostic centres from across the province were found to be smear-negative at the reference laboratory.

Drug susceptibility tests were positive in samples. One sample grew *Mycobacterium* other than Tuberculosis (MOTT) and hence was excluded from further analysis. Out of the remaining 130 patients, the isolates from 115 (88.4%) patients were fully susceptible to all the 1st line drugs tested, while 15 (11.5%) patients showed resistance to one or more drugs. Resistance to Isoniazid (H) alone or in combination with other drugs was seen in 12 (9.2%) patients. Similarly, resistance to Streptomycin (S), Rifampicin (R), Ethambutol (F) and Pyrazinamide (P) was seen in 8 (6.1%), 4 (3%), 1 (0.76) and 6 (4.6) respectively (Table I).

Resistance to one drug was seen in 10 (7.6%) patients, 2 drugs in none of the patients, 3 drugs in 4 (3%)

Table I: Summary of susceptibility reports-387 samples.

Resistant to	Frequency	Percent
Streptomycin 10 ug	08	6.1 %
Isoniazid 1 ug	12	9.2 %
Rifampicin 5 ug	04	3.0 %
Ethambutol 10 g	01	0.76 %
Pyrazinamide 100 g	06	4.6 %
MDR	02	1.5 %

Table II: Resistance pattern of *Mycobacterium* TB (42 resistant samples).

	Numbers	Percentage
Total Culture +ve	130	100.%
Fully sensitive	115	88.4 %
Any resistance	15	11.5 %
Resistance		
Only H	06	4.6 %
Only R	01	0.76 %
Only E	0	0 %
Only S	03	2.2 %
Only P	0	0 %
HE	0	0 %
HR	0	0 %
HP	0	0 %
HS	0	0 %
HSP	03	2.2 %
HEP	0	0 %
HRP	01	0.76 %
HRSP	01	0.76 %
HREP	0	0 %
HRSEP	01	0.76 %
Any H resistance	12	9.2 %
Any R resistance	04	3 %
Any HR resistance	02	1.5 %

Keys: *H=Isoniazid, R=Rifampicin, E=Ethambutol, S=Streptomycin, P=Pyrazinamide.*

patients, 4 drugs in one (0.76%) patients and one (0.76%) sample showed resistance to all the 5 drugs. Primary MDR was found in 2 (1.5%) patients (Table II).

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 2-41%. Overall, the median prevalence of primary MDR-TB was 1.4% ranging from 0-10%. Among the South East Asian Region (SEAR) countries, the prevalence or 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 9.8-36.6%. The median prevalence of primary MDR-TB was 2.5% significantly higher than the global mean of 1.4%.[3]

This study revealed culture positively of over 90% of all the smear-positive patients. The level of drug resistance to isoniazid, rifampicin, and MDR of 9.2%, 3%, and 1.5% respectively in previously untreated cases, as is evident from this study, is not as high as one would have expected, keeping in view that Karachi 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, *et al.* the primary resistance to isoniazid and rifampicin was found to be 11% and 15% respectively.[17] In another study, resistance was found to be even higher with H 25%, R 15%, E 12% and S 19%.[18] The relatively high percentage of resistance to individual drugs in studies from Pakistan 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.[22,23] There is a strong need to evaluate the primary resistance to anti-tuberculosis drugs in various provinces and the whole country.

Strong TB control programme with DOTS strategy can control 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 drug 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 programme.

CONCLUSION

This study shows that although the frequency of Primary MDR-TB was 11.5%, implementation of DOTS strategy is required to prevent the generation of resistant strains. A poorly functioning programme can create MDR-TB much faster than it can be treated, even if unlimited resources are available.

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