## SHORT COMMUNICATION

# Pattern of anti-tuberculosis drugs susceptibility in new and previously treated tuberculosis patients and environmental risk factors investigation

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**Abstract**: Resistance pattern both in newly and previously treated-TB patients and risk factors associated in spread of tuberculosis are investigated in the current study. A total 244 *Mycobacterium* tuberculosis isolates were used for drug-susceptibility test against four drugs. Environmental risk factors were assessed by using self-designed history proforma. Among 244 TB-isolates, 64% were categorized as MDR-TB in drug-susceptibility test. Male proportion was 51% while 32% belonged to 15-34 years age group and 49% were from city Lahore whereas majority of people (31%) was working on daily wages. Divergent drug-resistance pattern was obtained; RIF (68%), SM (52%), EMB (51%). INH showed only (27%) resistance against first-line anti-TB drug. Drug-resistance prevalence for two drug combination was highest (50%) for (INH+SM) and (INH+EMB) followed by (RIF+SM) (49%) whereas for three drugs combination (INH+RIF+EMB) and (INH+RIF+SM) the prevalence was almost same 50% and 49% respectively while 66% patients were categorized as previously treated and 34% as new TB cases. In drug susceptibility test, 71% were identified as MDR-TB among New TB cases, while 63% were identified as MDR-TB from previously treated cases. Surprisingly DST results displayed that percentage prevalence of MDR-TB both in newly and previously treated cases was almost same.

**Keywords**: *Mycobacterium* tuberculosis, multi-drug resistance, drug susceptibility test, drug resistance.

## INTRODUCTION

Ghulab Devi Chest hospital is located in the city of Lahore, the provincial metropolitan with a population of 12 million. Its shanty towns have a very high incidence of tuberculosis as up to six persons living in a single room. Healthy people are obliged to live with tuberculosis patients which ultimately results in spread of tuberculosis. It is alarming to note that the patients of multi drug resistant TB are transmitting the bacilli to healthy people which is difficult to treat. Although Ghulab Devi Chest hospital treats patients from other parts of the province but 49% patients in the hospital belong to the Lahore city (table 1).

The level of exposure to a TB infected individual with consistency is basic environmental parameter associated with the tuberculosis dispersal. Overcrowded and low housing quality is linked with poverty and this ultimately leads to high vulnerability for the disease (McHardy and O'Sullivan (2004); Kunimoto *et al*., 2004). Congested living style is a tuberculosis transmission risk factor (Hawker *et al*., 1999; Beggs *et al.,* 2003). In communities where people suffering to tuberculosis live, there crowded houses are subjected to at risk of more *M. tuberculosis* exposure and this risk aspect increases if the air circulation is not proper (Beggs *et al.,* 2003; Lienhardt 2001). People who live and sleep near to the infected person with TB are at more risk to suffer the disease (Menzies *et al.,* 1999; Singh *et al*., 2005; Fennelly *el al.,* 2004). Proper diagnosis of TB is a bit lengthy process, during this time period the persons in contact with the TB carrier are at continued risk of TB infection (Long *et al.,* 1999).

In human, tuberculosis was detected since long time ago (Lawn and Zumla, 2011). Tuberculosis is a transferable disease to other peoples, the cause of which is *Mycobacterium* tuberculosis (MTB) (WHO, 2016). Along with the lungs, it can influence other body parts as well. Majority of the infections do not show the sign that is known as the latent tuberculosis. Almost 10% of the latent infection leads to the development of the active tuberculosis which if not treated can kill half of the infected people. Persistent cough with stains of blood sputum, loss of weight, sweating at night, and fever are the classic signs of tuberculosis (CDC, 2016).

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Air is the medium of transfer for bacilli of active tuberculosis, its bacilli perforate to lungs when an infected person coughs, sneezes or speaks where bacilli stay either at preliminary infected site or the circulatory fluids take them away to other parts of body (Mustafa *et al*., 2005; Ulrichs *et al.,* 2005). Active tuberculosis can be diagnosed by the X-rays and examination of the sputum under microscope and by the culturing of the body fluids (Konstantinos, 2010).

Spread of tuberculosis can be hindered by the early and proper diagnosis, suitable treatment and vaccinations (Hawn *et al.,* 2014; Harris, 2013; WHO, 2008). The people living in house, workplace and in social contact with tuberculosis vicinity are more vulnerable to active tuberculosis (WHO, 2002). Resistance to the treatment against first line anti-biotic is a thriving issue which is causing an increase in the multiple drug resistance (MDR-TB) (WHO, 2016).

One third population of the world is considered to be influenced by tuberculosis (WHO, 2016). The rate of new cases of infection with tuberculosis is 1% each year (WHO, 2002). 9.6 million active TB cases ultimately lead to 1.5 million causalities in the year 2014. The rate of mortality in developing countries is greater than 95% (WHO, 2016). Improper and undesirable utilization of TB regimens can ultimately lead to multi drug resistant TB (CDC, 2006). MDR-TB is declared a hazard in reducing tuberculosis spread because of high cost of treatment and difficult to treat the strains of MDR-TB (Goble *et al.*, 1993; Mahmoudi and Iseman, 1993). On a global scale 4, 40,000 MDR-TB cases per year are declared which is parallel to 3.6% of accumulated newly identified TB cases. According to WHO calculations, Pakistan is among 27 MDR high burden TB countries. Roughly around 2-3.2% freshly detected and 35% former patients from Pakistan incorporate MDR-TB (Javaid *et al.,* 2008). Remedy momentum for MDR-TB is slow in comparison to drug susceptible TB (Rao *et al.,* 2009; WHO, 2008). The current study reveals drugs susceptibility pattern of new and previously treated patients, analysis of clinical parameters and their characterization.

## MATERIALS AND METHODS

The sampling and drug sensitivity test was performed from 2011 to 2013 at in programmatic management of drug resistant TB (PMDT) Unit, Ghulab Devi Chest hospital Lahore, Pakistan. The environmental risk factors responsible for tuberculosis transmission were evaluated in local population by filling clinical history proforma. Drug sensitivity test was performed by using Lowenstein Jensen medium for two hundred forty four samples (n=244). Drug sensitivity test (DST) performed by following the standard procedure was recommended by WHO guidelines. Final concentration in solid LJ-medium of drugs was 0.2µg/mL for Isoniazid, 40µg/mL for Rifampicin, 2.0µg/mL for ethambutol and 4µg/mL for streptomycin. After 3 to 4 weeks of incubation at 37ºC, the medium with growth was compared with control.

A sample was declared to be resistant when there was more than 1% growth of *Mycobacteria* in comparison to the drug free medium. TB patients, who were not improving for negative smear after taking the anti-tuberculosis (ATT) drug, were screened for the MDR-TB by the DST method. When the isolates were resistant for isoniazid and rifampicin the sample was diagnosed as MDR-TB. Evaluation of the DST results was carried out by using WHO benchmarks viz. cured, treatment complete, treatment failure, deaths.

For categorical factors relating to the patient's relating data like age, gender, area, occupation, sputum result at the beginning of treatment, type of drug resistance i.e. DST out comes, the percentages as well as frequency was computed in tabular form.

***Ethical approval***

Ethical committee of University of the Punjab, Lahore, Pakistan has approved the current study. The purpose of the research work was explained and written consent from the patients or their caretakers, or next of kin was obtained.

## RESULTS

In total, out of 244 pulmonary TB cases 155 (64%) were identified as MDR-TB and 12 (5%) were identified XDR-TB on the basis of drug susceptibility tests. Ninety (37%) had completed the treatment course, seventy four (30%) were effectively cured, treatment failed for seventy (27%) and only ten (4%) expired (table 1). Sputum test, before the start of treatment was positive for 143 (57%) and negative for 101(41%) samples (table 1). None of the samples was positive for infection after HIV screening (n=244). Number of male patients was 124 (51%) and 120 (49%) were females. Majority of the patients belonged to economically productive age group, as 31% belonged to15-24 years, 32% from 25-34 years while 16% belonged to 35-44 years. Majority of the patients (49%) were from the city of Lahore, (16%) of the patients came from Kasur, (12%) from Faisalabad and (5%) were from Toba Take Singh (table 1), rest of them belonged to other parts of the Province of Punjab. Most of the patients were causal workers working on daily basis as a labor (31%) and their monthly income was less than 5000Rs. While (19%) were still studying, 27% of the patients were house wives and rest of them belonged to other occupations.

160 (66%) were positive for previous treatment and eighty four (34%) were new cases without having any prior anti-tuberculosis treatment table 2. In new un-treated

**Table 1**: Drug susceptibility parameters of tuberculosis samples

| Sr. No | Drug susceptibility Parameters | | Number (n=244) | %age |
|---|---|---|---|---|
| 1 | Gender | Female | 120 | 49 |
| | | Male | 124 | 51 |
| 2 | Age | 5-14 | 14 | 6 |
| | | 15-24 | 75 | 31 |
| | | 25-34 | 78 | 32 |
| | | 35-44 | 40 | 16 |
| | | 45-54 | 24 | 10 |
| | | 55-64 | 7 | 3 |
| | | >65 | 6 | 3 |
| 4 | Area | Lahore | 120 | 49 |
| | | Kasur | 40 | 16 |
| | | Faisalabad | 30 | 12 |
| | | T.T. Singh | 11 | 5 |
| | | Jhang | 8 | 3 |
| | | Sargodha | 5 | 2 |
| | | Sheikhupora | 5 | 2 |
| | | Others | 25 | 10 |
| 5 | Occupation | Labour | 75 | 31 |
| | | Student | 45 | 19 |
| | | House wife | 65 | 27 |
| | | Driver | 6 | 3 |
| | | Shopkeeper | 5 | 2 |
| | | Tailor | 5 | 2 |
| | | Others | 43 | 18 |
| 6 | HIV co-infection | Positive | 0 | 0 |
| | | Negative | 244 | 100 |
| 7 | Tuberculosis treatment history | Completed | 90 | 37 |
| | | Failed | 70 | 27 |
| | | Cured | 74 | 30 |
| | | Expired | 10 | 4.0 |
| 8 | Sputum result at the beginning of treatment | Negative | 101 | 41 |
| | | Positive | 143 | 57 |
| 9 | Type of drug resistance | MDR | 155 | 64 |
| | | XDR | 12 | 5 |
| | | Gene Xpert/Rif resistance | 75 | 31 |
| 10 | Previously treated | Positive | 160 | 66 |
| | | Negative | 84 | 34 |

**Table 2**: Drug resistance pattern comparison between new and previously treated TB cases

| Sr. No | Pattern of resistance | No. of new un-treated cases (n=84) | Resistance (%) in new cases | No. of previously treated cases. (n=160) | % of resistance in previously treated cases |
|---|---|---|---|---|---|
| 1 | One or more drugs resistance | 64 | 76 | 102 | 64 |
| 2 | One drug resistance | 2 | 2 | 0 | - |
| 3 | Two drug resistance | 2 | 2 | 9 | 6 |
| 4 | Three drug resistance | 8 | 10 | 17 | 11 |
| 5 | Multi-drug resistance | 60 | 71 | 101 | 63 |

cases sixty four (76%) had the resistance for any drug and 71% were MDR table 2. The proportion of resistance for one and two drugs in new un-treated cases was 2% each and 10% for three drugs resistance. Resistance rate for previously treated cases was high 102 (64%); they had resistance to any drug. Resistance for two and three drugs was 6% and 11% respectively. 101 (63%) were diagnosed as having resistance to multi-drugs.

**Table 3**: Pattern of resistance to four anti tuberculosis drugs in (n=244) TB samples

| Sr. No | Pattern of drug resistance | | %age of resistant isolates (n=244) |
|---|---|---|---|
| 1 | Any drug resistance | | 83 (34) |
| 2 | One drug resistance | Isoniazid | 67 (27) |
| | | Rifampicin | 165 (68) |
| | | Streptomycin | 126 (52) |
| | | Ethambutol | 125 (51) |
| 3 | Two drug resistance | INH+Rif | 64 (26) |
| | | INH+SM | 122 (50) |
| | | INH+EMB | 122 (50) |
| | | SM+EMB | 103 (42) |
| | | Rif+SM | 120 (49) |
| 4 | Three drug resistance | INH+Rif+EMB | 123 (50) |
| | | INH+Rif+SM | 120 (49) |
| | | INH+SM+EMB | 102 (42) |

*INH=isoniazid; Rif= Rifampicin, SM= Streptomycin, EMB=Ethambutol.

Complete results of DST for (n=244) samples in detail are illustrated in table 3. The 165 (68%) of the samples showed resistance to single drug rifampicin. The two drug co-resistance was same for isoniazid & streptomycin (50%) and isoniazid & ethambutol (50%); followed by rifampicin and streptomycin (49%). For streptomycin and ethambutol it was (42%). In drug susceptibility testing there was high co-resistance in isoniazid, rifampicin and ethambutol (50%); followed by (49%) for isoniazid, rifampicin and streptomycin and (42%) for isoniazid, streptomycin and ethambutol the first line anti-tuberculosis drugs (table 3). Our study distinctly showed that the rate of prevalence of multi drug resistance tuberculosis in newly reporting and previously treated patients is more or less same.

## DISCUSION

TB is multifactorial malfunction which involved host related environmental risk factors like the extent of exposure to healthy person from infected one, crowding, poor ventilation, malnutrition and urbanization play important role in development of the lung disease. Dispersal of *M. tuberculosis* to a healthy person is more likely if there is more crowding with inadequate ventilation system in surrounding environment of the infected person. The percentage of MDR-TB restoration to healthy ones is 70-90% (Sampath, 2008) and death rate had risen to 60% (Irfan *et al.*, 2006) but these proportions are less in patients with HIV infection (Seung *et al.,* 2009). In current study before DST performance (n=143) samples were sputum positive and 101were negative. The new tuberculosis cases without any prior antidote were 84 and from these 76% were resistant to any drug, 2% for two drug combination and 10% resistance was calculated for three drug integration and surprisingly 71% were recognized as MDR after DST outcomes. The number of patients on anti-TB drug was (n=160) and categorized as previously treated ones. For any drug resistance 102 (64%) cases were identified in previously treated cases and for two and three drugs combination the percentage was 6% and 11% respectively, 63% were recognized as multi drug resistant. In current research work treatment was completed for 37% of cases; (30%) were cured effectively. Treatment failure rate was 27% which is a matter of concern as failure can result in emergence of more MDR cases. The expired patients under current study were 4%. Precautionary measures and actions must be taken for control and treatment of MDR-TB to stop the spread of disease. Proportion of male and female cases for current study was almost same i.e. 51% and 49%. Majority of TB cases belonged to 15-45 years of age group. Economy of the country can be influenced by tuberculosis infection in the work force of productive age group. Highest proportion of TB cases (49%) was from Lahore, (16%) were from Kasur and (12%) were from Faisalabad. In comparison to rural areas the urban localities have more incidence of tuberculosis prevalence (Hunter and Thomas, 1984). Occupation of most of the patients was working on daily wages (31%) and their earning was less than 5000 rupees (48 $) per month. The matter of concern for current study is that (19%) of the cases were student and 27% were the house wives.

This study revealed that, both for new and previously treated patients, high proportion of TB cases was calculated to be resistant to one or more drugs. Also DST results showed high percentage of multi drug resistant cases for both groups. Collectively DST results for all the samples (n=244) included high drug resistance for rifampicin (68%). For two drugs combination it was low for INH & Rif where it was (26%). Similarly there was low percentage of resistance (42%) in three drugs combination (isoniazid, streptomycin and ethambutol) as compared to other combinations (table 3).

## CONCLUSION

Surprisingly calculated DST results for undersigned study displayed that MDR-TB prevalence was on parallel scales

for newly and previously treated case. Majority cases belong to productive age group which can influence the country's economy which highlights that the patients should be monitored properly under strict supervision so that they complete the drug treatment course to avoid the spread of drug resistant tuberculosis in healthy population.

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