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# PRIMARY AND ACQUIRED DRUG RESISTANCE AMONG RIFAMPICIN RESISTANT TUBERCULOSIS CASES DIAGNOSED ON GENEXPERT

**Muhammad Kashif Munir[1*], Saba Shamim[2], Muhammad Amer Nazir[3], Sana Rehman[4], Ayesha Aftab[5], Asif Hanif[6], Muhammad Saqib Saeed[7]**

[1*]Senior Research Officer, HRI-NIH TB Research Centre KEMU/ Mayo Hospital, & PhD Scholar at The University of Lahore, Lahore, Pakistan
[2]Associate Professor, IMBB The University of Lahore, Lahore, Pakistan
[3]Assistant Professor, Department of Applied Sciences, FEST, Hamdard University, Karachi, Pakistan
[4] Research Officer, NIH-HRI CRC National Institutes of Health Islamabad, Pakistan
[5]Associate Professor, Department of Pharmacology, Al-Nafees Medical College and Hospital, Islamabad, Pakistan
[6]Assistant Professor, Department of Pulmonology KEMU/Mayo Hospital, Lahore, Pakistan
[7]Professor (Retired), Department of Pulmonology KEMU/Mayo Hospital, Lahore, Pakistan

***Corresponding Author:** Dr. Muhammad Kashif Munir
*Senior Research Officer, HRI-NIH TB Research Centre KEMU/ Mayo Hospital, Lahore, Pakistan, Email: munir_gemini81@gmail.com

**Abstract**
Drug resistance tuberculosis may be primary or acquired based on treatment history of prior tuberculosis and it is necessary to observe the patterns of resistant among both groups. Purpose of this research was to compare the patterns of drug resistance among primary and acquired drug resistant tuberculosis patients found rifampicin resistant by Xpert MTB/RIF Assay. This cross sectional comparative study was conducted through collaboration among IMBB The University of Lahore and HRI-NIH TB Research Centre Mayo Hospital Lahore during. Pulmonary TB patients provided sputum samples which were processed for Xpert MTB/RIF Assay, smear preparation for Auramin staining, culture and drug susceptibility on Lowenstein Jensen medium. Data of 170 patients was analyzed presently consisting of 86 (50.6%) primary and 84 (49.4%) acquired drug resistance cases, 90 (52.9%) males and 80(47.1%) were females. Presently 133 (78.3%) cases were found to have multidrug resistant tuberculosis. Around 2.9% cases remained susceptible to all drugs while 6.4% cases remained susceptible to rifampicin by drug proportion method. Higher rates of drug resistance as well as multidrug resistant tuberculosis were endorsed in acquired drug resistance cases as compared to primary drug resistance cases primarily diagnosed by Xpert MTB/RIF Assay.

**Keywords:** GeneXpert, Multidrug resistant, Acquired & Primary resistance, Diagnosis, Susceptibility.

## Introduction

Tuberculosis (TB) is a contagious and communicable disease and remains a major cause of illness in developing and underdeveloped countries. It is also amongst top ten causes of global deaths and leads in casualties due to single infectious mediator which is ranked above *Human immunodeficient virus* (HIV).[1] Spread of TB is also easy as compared to HIV, through coughing and expelling *Mycobacterium tuberculosis* (MTB) bacilli in the air and inhaled by healthy individuals to acquire infection. Burden of TB varies among different countries with a minor number of 5 to a big number of 500 new cases per 100000 inhabitants. Pakistan is facing a high incidence of 263/100,000 Population. [1]

Appearance of drug resistance among TB patients from late 1980's triggered to declared as Global emergency in 1993 by WHO and in 2001 by Pakistan.[2] Developed countries had overcome the challenge by implementing the standards while developing countries remained either unable or delayed to achieve the minimum targets required to control the disease. Infection control measures on the other hand are hard to implement due to various social challenges in Pakistan. [3]

Resistance of MTB strains against two major first line anti TB drugs i.e. isoniazid and rifampicin is said to be the multidrug resistant (MDR) TB. The strains of MDR TB are significant cause of morbidity and mortality around the globe.[4] Factors attributing in occurrence of MDR TB include programmatic deficiencies, non-adherence of patients, default, hiding the history of previous TB treatment or ignorance of previous treatment history by physician etc.[5] Emerging drug resistance is becoming more challenging from last decade. Few strains of MTB have started to show resistance against broad spectrum second line drugs. [5] More severe type of drug resistant TB is extensively drug resistant (XDR) TB which comprises additionally resistant to one of three second line injectable (capreomycin, kanamycin or amikacin) and flouroquinolones.[6]

The patients failing to respond the treatment are expected to acquire drug resistance. [7] Previously, in many programs multiple iterations of first line ATT were prescribed even in cases of unsuccessful causing amplification of resistance, which is also responsible to increase the resistance against more number of drugs.[8] This type of drug resistance among TB patients is called acquired drug resistance while any patient gets infection from already resistant strain of MTB is called primary drug resistant patient.

An upsurge of drug resistance among TB patients reflects many deficiencies thus create difficulties for physicians in diagnosing drug resistant TB cases. [9] Rapid diagnosis of rifampicin resistant patients became possible with the invention of GeneXpert technology and WHO has introduced it for primary isolation of drug resistant TB patients from susceptible patients. [10] Therefore it is being used for initial diagnosis of drug resistant TB to start treatment accordingly.[11]

Early detection and treatment of DR-TB are the most priority areas of NTP, therefore a high enrolment of drug resistant TB cases is observed on >29 programmatic management of drug resistant TB (PMDT) sites since 2016 in Pakistan. [12] Treatment failure is an important feature to observe the outcome and perceived by positive sputum smear or culture for acid fast bacilli after 5 months of treatment due to slow growing organism. Similarly TB cases susceptible to rifampicin and resistant to other first line TB drugs may grieve from treatment failure. [5]

Local studies show various degree of resistant patterns among TB patients as compared to the data published by WHO while such studies lack systematic sampling. Further rifampicin resistant TB patients are treated according to protocols of MDR TB patients without susceptibility testing. Therefore, patterns of drug resistant are important to observe especially rifampicin resistant cases diagnosed by GeneXpert. Further differences among primary and acquired drug resistance patterns are also of great importance. Keeping in view this study was undertaken to compare the drug resistance patterns among primary and acquired drug resistant TB patients diagnosed rifampicin resistant by Xpert MTB/RIF Assay.

## Material and Methods

This comparative cross sectional study was conducted through collaboration among IMBB The University of Lahore and HRI-NIH TB Research Centre Mayo Hospital Lahore. A sample size of 178

(89 in each group) patients were enrolled in this study. Pulmonary TB patients provided sputum samples for Xpert MTB/RIF Assay were processed according to the manufacturer's directions. [13] In MTB detected cases rifampicin susceptibility may be given as sensitive, resistant or intermediate. All the pulmonary samples obtained from adult patients of both genders and found rifampicin resistant by testing through Xpert MTB/RIF Assay with or without history of ATT were included in present study.

Ethical approval was obtained from King Edward Medical University through memo No. 438/RC/KEMU. After taking a written informed consent sputum samples from patients were collected individually in sterile sputum containers. Patients were divided in two groups on the base of previous treatment history in primary and acquired resistance groups. A pre-designed questionnaire was used to collect the demographic information, history and other required information.

**Isolation of MTB**

Modified Petroff's Method was used to decontaminate and concentrate the MTB in sputum samples and inoculated on Lowenstein Jensen (LJ) media. Existence of even one colony was reported as culture positive, however actual colony counts were reported if there is less than 50 colonies, more than 50 and less than 100 colonies 1+, 100 to 200 colonies 2+ and more than 200 colonies were reported as 3+. Known strain of American type culture control (ATCC) was used test quality of LJ media.

**Staining and Microscopy of Smears**

For auramine staining, protocol followed by Saeed et.al. was followed. [14] Stained smears were kept in the dark and scanned as soon as possible using fluorescence. Acid-fast bacilli appear bright green fluorescent against the dark background material and reported as follows in Table 1.

**Table 1: Criteria for reporting Acid Fast Bacilli in Each Smear**

| IUATLD/WHO scale (1000x field = HPF) Result | Microscopy system used | | |
|---|---|---|---|
| | Bright-field (1000x magnification: 1 length = 2 cm = 100 HPF) | Fluorescence(200–250x magnification: 1 length = 30 fields =300 HPF) | Fluorescence (400x magnification: 1 length = 40 fields =200 HPF) |
| Negative | Zero AFB / 1 length | Zero AFB / 1 length | Zero AFB / 1 length |
| Scanty | 1–9 AFB / 1 length or 100 HPF | 1–29 AFB / 1 length | 1–19 AFB / 1 length |
| 1+ | 10–99 AFB / 1 length or 100 HPF | 30–299 AFB / 1 length | 20–199 AFB / 1 length |
| 2+ | 1–10 AFB / 1 HPF on average | 10–100 AFB / 1 field on average | 5–50 AFB / 1 field on average |
| 3+ | >10 AFB / 1 HPF on average | >100 AFB / 1 field on average | >50 AFB / 1 field on average |

**Drug Sensitivity Testing**

Prime isolation of MTB was achieved by LJ media. Drug containing media with recommended concentrations was then used to sub culture pure isolates of MTB. [5] Any of MTB isolates presenting 1% growth for rifampicin, isoniazid, ethambutol, ofloxacin, kanamycin, amikacin, capreomycin, and cycloserin while more than 10% growth on medium containing streptomycin and ethionamid as compared with control was labelled as resistant strain. Internal quality assurance is achieved by performing susceptibility of samples from previous batches while for external quality assurance NRL sends 30 Belgium strains each year and results are compared afterwards. Data was entered and analysed in statistical package for social science (SPSS) software.

**Results**

A total 8 cases were dropped due to incomplete info and data of 170 patients was analysed presently consisting of 86 (50.6%) primary and 84 (49.4%) acquired drug resistance cases. Male gender was pre-dominant consisting of 90 (52.9%) proportion while female were 80(47.1%) in this study making a female to male ratio of 1:1.12. Mean age of drug resistant patients remained to be 35.45±16.11 with a higher mean age of males as 37.64±15.79 as compared to mean age of females as 32.99±16.11. Demographic characteristics and initial findings of the patients are shown in Table 2. Most of the

patients (65.9%) were married and a high proportion of 57.1% patients were either illiterate or having primary education.

**Table 2: Demographic characteristics and Screening of Patients**

| Variables | | Gender | | | | Total (n=170) | |
|---|---|---|---|---|---|---|---|
| | | Male (n=90) | | Female (n=80) | | | |
| | | n | % | n | % | n | % |
| **Marital Status** | Married | 56 | 62.2 | 56 | 70.0 | 112 | 65.9 |
| | Unmarried | 34 | 37.8 | 24 | 30.0 | 58 | 34.1 |
| **Education** | Illiterate | 29 | 32.2 | 31 | 38.8 | 60 | 35.3 |
| | Primary | 26 | 28.9 | 11 | 13.8 | 37 | 21.8 |
| | Middle | 17 | 18.9 | 10 | 12.5 | 27 | 15.9 |
| | High | 15 | 16.7 | 25 | 31.2 | 40 | 23.5 |
| | Higher Above | 3 | 3.3 | 3 | 3.8 | 6 | 3.5 |
| **Smear Result** | Negative | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | Scanty | 5 | 5.6 | 4 | 5.0 | 9 | 5.3 |
| | 1+ | 36 | 40.0 | 23 | 28.7 | 59 | 34.7 |
| | 2+ | 28 | 31.1 | 40 | 50.0 | 68 | 40.0 |
| | 3+ | 21 | 23.3 | 13 | 16.2 | 34 | 20.0 |
| **Xpert Result** | Not Detected | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | Very Low | 18 | 20.0 | 19 | 23.8 | 37 | 21.8 |
| | Low | 43 | 47.8 | 39 | 48.8 | 82 | 48.2 |
| | Medium | 16 | 17.8 | 14 | 17.5 | 30 | 17.6 |
| | High | 13 | 14.4 | 8 | 10.0 | 21 | 12.4 |
| **Rifampicin Resistant** | | 90 | 100.0 | 80 | 100.0 | 170 | 100.0 |

History of household TB and MDR TB contacts were explored and found to be 64.7% and 24.1% respectively while history of ATT was present in 49.4% cases presently. History of smoking is an important factor and present among 38.2% of total respondents while predominant among male gender (61.1%) as compared to female gender and difference remained significant (p value <0.0001). Similarly diabetes was also present among 23.5% patients presented in Table 3.

**Table 3: Histories of Registered Drug Resistant Cases**

| Histories | | Gender | | | | Total (n=170) | |
|---|---|---|---|---|---|---|---|
| | | Male (n=90) | | Female (n=80) | | | |
| | | n | % | n | % | n | % |
| **History of TB Contact**** | | 54 | 60.0 | 56 | 70.0 | 110 | 64.7 |
| **History of MDR-TB Contact**** | | 18 | 20.0 | 23 | 28.7 | 41 | 24.1 |
| **History of TB Treatment** | | 42 | 46.7 | 42 | 52.5 | 84 | 49.4 |
| **History of smoking** | | 55 | 61.1 | 10 | 12.5 | 65 | 38.2 |
| **History of Diabetes** | | 20 | 22.2 | 20 | 25.0 | 40 | 23.5 |
| **If diabetes present, from how long? (years)** | **Nil*** | 70 | 77.8 | 60 | 75.0 | 130 | 76.5 |
| | **1-3** | 12 | 13.3 | 10 | 12.6 | 22 | 12.9 |
| | **4-6** | 4 | 4.4 | 4 | 5.0 | 8 | 4.8 |
| | **7-9** | 2 | 2.2 | 3 | 3.7 | 5 | 3.0 |
| | **≥10** | 2 | 2.2 | 3 | 3.7 | 5 | 3.0 |
| **Other Chronic Illness** | | 4 | 4.4 | 0 | - | 4 | 2.4 |

Patients having the history of ATT were considered as acquired drug resistance while directly infected with resistant strains were considered as primary drug resistant cases. An overall agreement rate of rifampicin resistant cases on Xpert MTB/RIF Assay was remained to be 93.5% as compared gold standard drug proportion method of susceptibility through culture. Further acquired drug resistant cases showed an agreement rate of 98.8% high as compared to the 88.4% by primary drug resistant cases with an insignificant difference (P-value >0.05). Patterns of drug susceptibility among acquired

and primary drugs resistant cases showed a significant difference (p-value <0.05) for isoniazid, ethambutol, ofloxacin and ethionamid as shown in Table 4.

**Table 4: Primary and Acquired Drug Susceptibility Patterns**

| Drug | Total N=170 | | History of TB Treatment | | | | P value |
|---|---|---|---|---|---|---|---|
| | | | Present (n=84) | | Absent (n=86) | | |
| | n | % | n | % | n | % | |
| **Isoniazid** | 139 | 81.8 | 79 | 94.0 | 60 | 69.8 | <0.0001 |
| **Rifampicin** | 159 | 93.5 | 83 | 98.8 | 76 | 88.4 | 0.248 |
| **Ethambutol** | 28 | 16.5 | 20 | 23.8 | 8 | 9.3 | 0.011 |
| **Streptomycin** | 47 | 27.6 | 28 | 33.3 | 19 | 22.1 | 0.103 |
| **Kanamycin** | 1 | 0.6 | 1 | 1.2 | 0 | 0.0 | 0.31 |
| **Ofloxacin** | 39 | 22.9 | 25 | 29.8 | 14 | 16.3 | 0.037 |
| **Ethionamid** | 13 | 7.6 | 10 | 11.9 | 3 | 3.5 | 0.04 |
| **PAS*** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | - |
| **Cycloserin** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | - |
| **Amikacin** | 1 | 0.6 | 1 | 1.2 | 0 | 0.0 | 0.31 |
| **Capreomycin** | 1 | 0.6 | 1 | 1.2 | 0 | 0.0 | 0.31 |

**para-amino salicylic acid*

A total of 139 cases were isoniazid resistant by drug proportion method of which 133/159 showed an agreement rate of 83.3% that resistance to rifampicin confers resistance to isoniazid also while this rate further decrease to 78.3% considering rifampicin resistance through Xpert MTB/RIF Assay. Thus these 133 (78.3%) cases were found to be MDR TB cases by definition in this study. One resistant case for each of amikacin, kanamycin and capromycin was revealed while 2 (1.6%) cases were recorded as XDR TB cases.

Different Combinations of first line anti-TB drug resistance pattern was explored in primary and acquired drug resistant cases. A total of 5(2.9%) rifampicin resistant cases by Xpert MTB/RIF Assay were found to be sensitive on culture in present study. Mono drug resistance against isoniazid and rifampicin was shown to be high among primary drug resistant cases having no history of ATT, while patterns show a higher proportion in acquired drug resistant cases as presented in the table 5 below.

**Table 5: Combination of First Line Anti TB Drug Resistance Pattern on Culture.**

| Drug Combination | History of TB Treatment | | | | Total N=170 | |
|---|---|---|---|---|---|---|
| | Present (n=84) | | Absent (n=86) | | | |
| | n | % | n | % | n | % |
| **No Resistance** | **1** | **1.2** | **4** | **4.7** | **5** | **2.9** |
| **Isoniazid Only** | **0** | **0.0** | **6** | **7.0** | **6** | **3.5** |
| **Rifampicin Only** | **4** | **4.8** | **22** | **25.6** | **26** | **15.3** |
| **I+R** | **43** | **51.2** | **33** | **38.4** | **76** | **44.7** |
| **R+E** | **1** | **1.2** | **0** | **0.0** | **1** | **0.6** |
| **I+R+E** | **11** | **13.1** | **2** | **2.3** | **13** | **7.6** |
| **I+R+S** | **15** | **17.9** | **13** | **15.1** | **28** | **16.5** |
| **I+R+E+S** | **9** | **10.7** | **6** | **7.0** | **15** | **8.8** |

**I = Isoniazid, R = Rifampicin, E = Ethambutol, S = Streptomycin**

## Discussion

Presently, a total of 133/170 (78.3%) rifampicin resistant cases by Xpert MTB/RIF Assay showed simultaneous isoniazid and rifampicin resistance by standard drug proportion method and proved the definition of MDR TB. Similarly, an agreement rate of 83.3% for the cases resistance to rifampicin conferred resistance to isoniazid also on the basis of drug proportion method alone whereas 5(2.9%) rifampicin resistant cases by Xpert MTB/RIF Assay were found to be sensitive on culture in present study. These findings are in concomitant with the findings of previous study that presented an

agreement rate of 81.25% rifampicin resistance cases conferring the simultaneous isoniazid resistance to prove MDR TB. [15] Present results are not comparable with the study that revealed 90% chances of the coexistence of isoniazid resistance with rifampicin resistance. [16]
An agreement rate of rifampicin resistance on Xpert MTB/RIF Assay also showing resistance on standard proportion method remained to be 93.5% in this study. Phenotypic drug sensitivity (DS) of TB cases using culture technique is still gold standard on the other hand rifampicin DS has not been considered to be optimized but conventionally reflected as reliable and accurate further endorsed by WHO. [17] The study reported an agreement rate of Xpert MTB/RIF Assay and drug proportion method in disputed and undisputed groups as 78.7% and 96.7% respectively. [17]
An overall agreement rate of rifampicin resistance between both techniques remained to be 93.5%. A significant difference ($p < 0.05$) of primary and acquired drug resistance cases was found for rifampicin, isoniazid, ethambutol, ofloxacin and ethionamid while it was not significant for rest of first and second line anti TB drugs tested in this study. Results are not in agreement with various studies showing highly significant difference of drug resistance among primary and acquired drugs though the these studies lack systemic sampling [5,9] further inclusion criteria a bit different from present study as it included the cases of rifampicin resistance on Xpert MTB/RIF Assay.

American Thoracic and Infectious Disease societies consider highest mono resistance to isoniazid and recommend rifampicin, ethambutol, pyrazinamide and latest genera of fluoroquinolone for 6-9 months. Mono resistance to isoniazid on the other hand is considered rare but shown to be high in various regions around the globe as is in this study whereas removal of rifampicin from treatment increases the therapeutic time duration which is further found to be associated with rifabutin and rifapentine. [18] Thus various patterns of first line TB drug resistance create hurdles in setting of treatment regime whilst it is also recommended to use as many first line drugs as possible. [19]

Contrary to present findings a recent study on MDR TB patients reported presented high proportion 56.2% females, lesser frequency of 49.8% patients below age of 40 years and as many as 78.3% unmarried patients. [20] Sign and symptoms of patients presented in this study are the most common and also comparable with the guidelines of NTP Pakistan. [12]
In conclusion it is revealed that 21.7% rifampicin resistant cases by Xpert MTB/RIF Assay were not having MDR TB by gold standard further 2.9% of the cases were sensitive to all of the first line and second line anti TB drugs tested in this study. Although primary drug resistance is direct transfer of infection from resistant strains by even mechanism but higher rates of drug resistance as well as MDR TB were endorsed in acquired drug resistance cases as compared to primary drug resistance cases. Further treatment of rifampicin resistant TB cases diagnosed by Xpert MTB/RIF Assay similar to MDR TB patients could not be justified under the light of present results.

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**Ethics Approval:** Ethical approval for this study was obtained from Institutional Review Board of King Edward Medical University and it is certified that study was performed in accordance with ethical standards as laid down in 1964 Declaration of Helsinki and its later amendments.

**Author's contribution:** All authors have significant contribution in this study. MKM: Conceptualization, MKM & SR: Methodology, Design questionnaire and Data curation, MAN & AA: Literature search and Writing- Original draft preparation. AH & MSS: Visualization, Investigation. SS: Supervision, SR & AA: Software, Validation, MAN & SR: Analysed the data, MKM & MAN: Writing- Reviewing and Editing.

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