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*Original Research Article*

# Frequency of Drug Resistance Among Tuberculosis Relapse and Factors Contributing to Tuberculosis Relapse

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***Abstract***: ***Background:*** Multidrug-resistant Tuberculosis (MDR-TB) is a significant global health threat, with Pakistan ranking 5th in TB burden. Limited resources hinder the diagnosis and monitoring of MDR-TB cases. The GeneXpert tool has emerged as a crucial method for diagnosing pulmonary Tuberculosis worldwide. This study aimed to investigate predictors of relapse in TB patients and the prevalence of drug resistance. ***Objective:*** To identify factors associated with relapse among cured TB patients and to determine the frequency of drug resistance. ***Methodology:*** We randomly selected 355 pulmonary TB cases from the outpatient department of Ghulab Devi Chest Hospital, Lahore, between August and December 2022. Participants had previously undergone successful treatment. A structured questionnaire was used for interviews following the confirmation of AFB in sputum through microscopy. The ZN staining method was applied, and the GeneXpert was utilized for molecular detection of MDR-TB. ***Results:*** Among the 355 patients, 23 (6.5%) were diagnosed with MDR-TB, comprising 11 males (47.8%) and 12 females (52.2%). The percentages of MDR-TB cases based on treatment duration were as follows: 6 months = 5.9%, 7 months = 1.9%, 8 months = 13.3%, 9 months = 11.1%, with 30 cases (36%) indicating relapse. GeneXpert results showed 245 (69.0%) MTB detected, 56 (15.8%) not detected, 31 (8.7%) indeterminate, and 23 (6.5%) rifampicin resistance detected. ***Conclusion:*** Relapse is a significant factor contributing to the failure of TB control programs. Monitoring comorbidities and adjusting treatment regimens are essential for improving outcomes.

*Keywords*: ziehel neelsen (ZN) smear microscopy, GeneXpert, multi-drug resistance, MDR; Lowenstein jensen media, (LJ)Tuberculosis; TB

## INTRODUCTION

Tuberculosis, caused by Mycobacterium tuberculosis (MTB), is one of the most common diseases affecting humans worldwide1. Multidrug-resistant tuberculosis (MDR-TB) is a well-recognized global health threat that emerged due to the development of resistance against Isoniazid (INH) and Rifampicin (RIF), the two best-known first-line antituberculosis drugs. It has been reported that up to 20% of TB cases across the globe are MDR-TB2. In Asia, Central Asia has the highest prevalence of MDR-TB, while in Europe, Eastern Europe has more cases of MDR-TB as compared to the other regions.

Disturbingly, a 60% prevalence rate of MDR-TB has been reported from India, China, and Russia2. In addition to that, extensively drug-resistant tuberculosis (XDR-TB), which showed resistance to quinolones as well, has also been reported from various parts of the world3. Prompt and precise diagnosis of MDR-TB is very critical for the management of the infection; it also restrains the dissemination of disease 3-5 Mutation in rpoB gene resided at rifampicin resistance determining region (RRDR) is associated with the resistance in MDR-TB against first-line drugs6, the mutation in cluster II region or N-terminal of the rpoB gene may be a factor involved in the development of resistance in MDR-TB 4-6 Development of antibiotic resistance leads to the treatment failure and emergence of MDR-TB 4-6

Mutation detection of the rpoB gene in MDR-TB through molecular assays has been well recognized

 

and has a wide range of applications in clinical diagnostic settings 4-5 The Xpert MTB/RIF (Xpert) is one of the This assay is a DNA amplification-based test that simultaneously detects the DNA of the bacterium and RIF resistance in a very short time. GeneXpert is a semi-automated instrument being used with GeneXpert(R) System (Cepheid, Sunnyvale, California, USA).

It is a nested real-time PCR in vitro diagnostic test for the detection of complex DNA of MTB and RIF resistance-associated mutations in the rpoB gene in sputum samples from patients at risk for rifampicin resistance (RR). Pakistan is a low-middle-income country and ranks 5th among nations with the highest TB burden across the globe. Pakistan is also listed among countries where MDR-TB is a significant challenge in health care settings. It is a nested real-time PCR in vitro diagnostic test for the detection of complex DNA of MTB and RIF resistance-associated mutations in the rpoB gene in sputum samples from patients at risk for rifampicin resistance (RR) gene8. Pakistan is a low-middle-income country and ranks 5th among nations with the highest TB burden across the globe. Pakistan is also listed among countries where MDR-TB is a significant challenge in health care settings 9.

Key reasons associated with the high prevalence of MDR-TB in the country include illiteracy, population, poverty, and inadequate monitoring and control measures. Studies from Pakistan have demonstrated the diagnostic accuracy of the GeneXpert assay in detecting rifampicin resistance in patients with clinical tuberculosis. Additionally, the Xpert MTB/RIF assay has been evaluated for diagnosing pulmonary TB in pediatric patients in local, resource-limited healthcare settings9. The present study was designed to estimate the occurrence of MDR-TB in pulmonary patients and identify associated risk factors for the infection using the GeneXpert(R) assay.

Retreatment pulmonary TB exclusively due to non-adherence to treatment with anti-TB drugs has been widely studied, chiefly in high-burden TB countries. The common factors accountable for default or failure of TB treatment have been classified as age, gender, marital status (personal), alcohol use, income, employment (behavioral and socio-economic), health system, co-morbidities, and community.

This study was conducted to understand the factors contributing to relapse and resistance, and subsequently plan new strategies for their control. The steps taken will eventually help control TB in Pakistan.

## Materials and Methods

It was cross sectional conducted between August 2022 to December 2022 in Outpatient Department of Pulmonology ward of Ghulab Devi Chest Hospital Lahore.

A total of 355 cases of pulmonary tuberculosis were selected randomly having a history of previously successful Anti-TB treatment. Standard questionnaire was designed, the selected cases after the confirmation of AFB in their sputum on microscopy were asked about their medical history, household, demographic & social characteristics. Information regarding BCG vaccination, weight, height, clinical symptoms of TB and co-morbid conditions, if any were also recorded.

International definitions were used for the outcomes of treatment. Relapse is a case who is again positive for TB after successful treatment declared cured based on microbiological reports (two sputum samples positive for AFB by direct smear, one smear and one culture positive from separate samples, or two cultures positive). Selected patients were asked to submit their sputum samples for Sputum smear microscopy. The smear was stained with ZN method using 1% Carbolfuchsin, 25% sulphuric acid and 0.3% methylene blue. A minimum of 100 oil fields were observed to declare negative smear. Smear was considered positive if it contains at least 3 AFB in observed 100 oil fields for this study.

The results were reported according to the WHO/International Union of against Tuberculosis and Lung Diseases (IUATLD) criteria where no AFB per 100 high power field is reported as negative, 19 AFB per 100 high power field is reported as actual count per 100 high power field, 10-99 per 100 high power fields is reported as 1+, 1-10 AFB per high power field in at least 50 fields is reported as 2+ and more than 10AFB per high power field in at least 20 fields is reported as 3+

**OUTCOME OF UNIVARIABLES;**

The mean of variables were as followed age of respondents was 38.15±16.071(13-80) years, income of respondents was 15749.30±1438.736 (0-90000) rupee, height ($m^2$) of respondents was 2.7416±0.34210 (2.31-3.35). Weight (kg) of respondents was 49.67±12.995 (23-90), positivity of sputum was 3.80±2.184(0-12) microorganisms, BMI ($Kg/m^2$) of respondents was 18.3710±5.20755(6.87-37.46)

As per as gene Xpert in 245(69.0%) MTB was detected, 56(15.8%) MTB was not detected, 31(8.7%) were

indeterminate and 23(6.5%) were rifampicin resistance
while 332(93.5%) were not resistant (Figure 1). 195(54.9) respondents were males, while 160(45.1%) were females (Figure 2), 319(89.9%) respondents were married, while 36(10.1%) were unmarried (Figure 3).

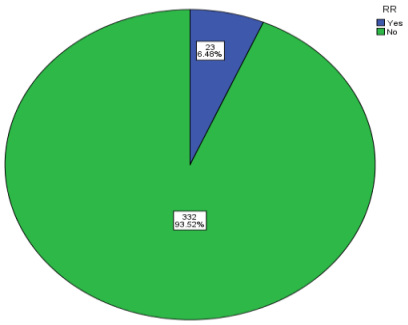


**Figure 1: Graph of Gene Xpert results showing Rifampicin Resistance**

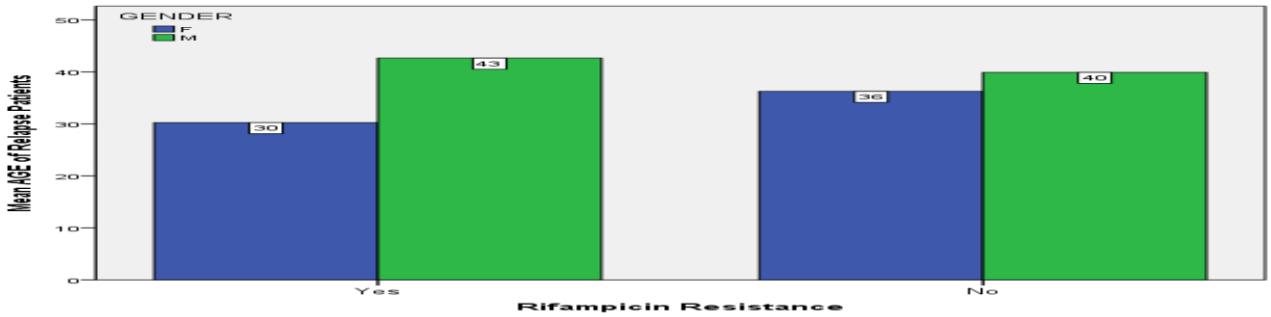


**Figure 2: Graph showing Rifampicin Resistance in age groups**

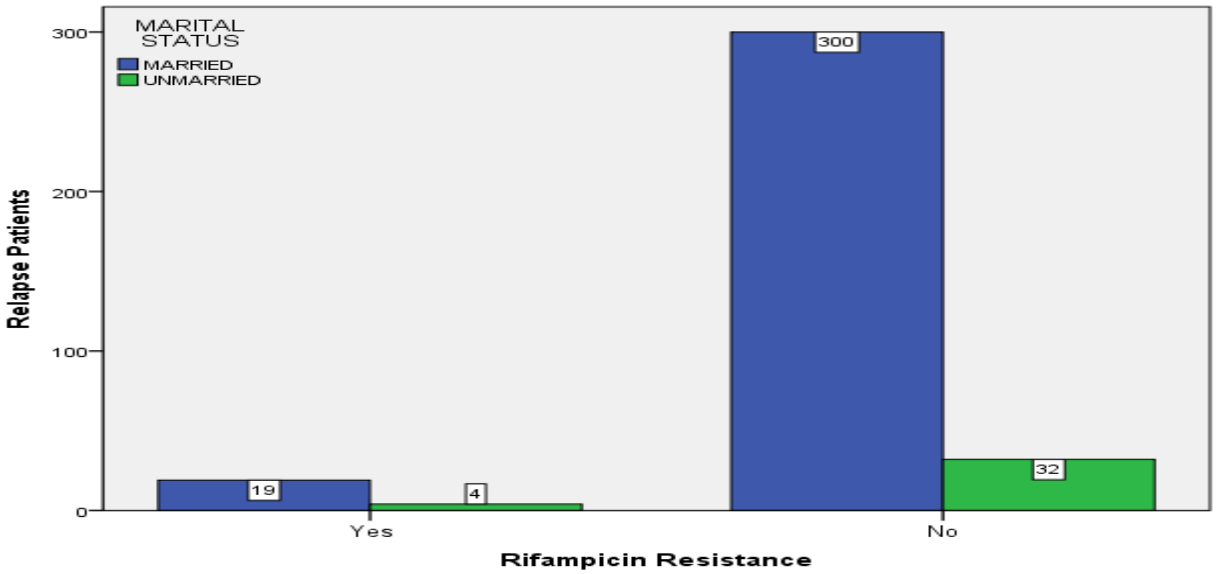


**Figure 3:Graph showing Rifampicin Resistance vs marital status**

**OUTCOME OF BIVARIATES;**

Table 1 shoes the percentage of variables that were rifampicin resistant were as follows, 7.5% females (P=0.497), 6.0% were married (P=0.273),12.2% were diabetic (P=0.165), 5.6% were hypertensive (P=1.000), 6.9% were HCV

positive (P=1.000),11.3% were HBV positive (P=0.131), 8.0% were having Renal disease (P=0.671),10.7% had joint disease (P=0.410), 3.8% were drug addict (P=1.000). 3.7% were heroine addict (P=1.000), 7.7% were addicted to charas (P=0.68), 5.1% were smoker (P=1.000), 9.7% had suffered drug side effects (P=0.439).

Patients who were rifampicin resistant and were having history of TB treatment were as followed; 5.9% of patients had 6 months of treatment, 1.9% of patients had 7 months of treatment,13.3% of patients had 8 months treatment, 11.1% of patients had 9 months treatment (P=0.103).

The mean of variables having rifampicin resistant; Age 36.17±16.615 years (P=0.543), income 20565.22±20362.234 rupee (P=0.096),BMI 18.1579±5.63828 KG/$M^2$(P=0.853), height 2.7667±0.30962 $m^2$(P=0.724), weight 49.65±14.615(P=0.994), gap of drug interruption1.9500±0.76194 months (P=0.082),

**Table 1: comparison of RR with vairables**

| variable | | RR+ | | RR- | | P value | Remarks |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | | |
| Gender | M | 11 | 5.6 | 184 | 94.4 | 0.479 | Not significant |
| | F | 12 | 7.5 | 148 | 92.5 | | |
| Marrital status | M | 19 | 6.0 | 300 | 94.0 | 0.273 | Not significant |
| | U | 4 | 11.1 | 32 | 88.9 | | |
| HTN | yes | 3 | 5.6 | 51 | 94.4 | 1.000 | Not significant |
| | no | 20 | 6.6 | 282 | 93.4 | | |
| HCV | yes | 2 | 6.94 | 27 | 93.1 | 1.000 | Not significant |
| | no | 21 | 6.4 | 305 | 93.6 | | |
| HBV | yes | 6 | 11.3 | 47 | 88.7 | 0.131 | Not significant |
| | no | 17 | 5.6 | 285 | 94.4 | | |
| DM | yes | 5 | 12.2 | 36 | 87.8 | 0.165 | Not significant |
| | no | 18 | 5.7 | 296 | 94.3 | | |
| Smoker | yes | 2 | 5.1 | 37 | 94.9 | 1.000 | Not significant |
| | no | 21 | 6.6 | 295 | 93.4 | | |
| Alcoholic | yes | 0 | 0.0 | 26 | 100 | 0.396 | Not significant |
| | no | 23 | 7.0 | 306 | 93.0 | | |
| Charas | yes | 2 | 7.7 | 24 | 92.3 | 0.687 | Not significant |
| | no | 21 | 6.4 | 308 | 93.6 | | |
| Heroine | yes | 1 | 3.7 | 26 | 98.3 | 1.000 | Not significant |
| | no | 22 | 6.7 | 306 | 93.3 | | |
| Drug abuser | Yes | 1 | 3.8 | 25 | 96.2 | 1.000 | Not significant |
| | no | 22 | 6.7 | 307 | 93.3 | | |
| Joint problems | yes | 3 | 10.0 | 25 | 89.3 | 0.410 | Not significant |
| | no | 20 | 6.1 | 307 | 93.9 | | |
| Renal Disease | yes | 2 | 8.0 | 23 | 92.0 | 0.671 | Not significant |
| | no | 21 | 6.4 | 309 | 93.6 | | |
| Drugs S/E | yes | 3 | 9.7 | 28 | 90.3 | 0.439 | Not significant |
| | no | 20 | 6.2 | 304 | 93.8 | | |
| H/O Ptb Treatment | 6M | 14 | 5.9 | 224 | 94.1 | 0.103 | Not significant |
| | 7M | 1 | 1.9 | 53 | 98.1 | | |
| | 8M | 6 | 13.3 | 39 | 86.7 | | |
| | 9M | 2 | 11.1 | 16 | 88.9 | | |

**Table 2: comparison of RR with Demographic features**

| Variable | RR+ | | RR- | | P value | Remarks |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| Age | 36.17 | 16.615 | 38.29 | 16.050 | 0.543 | Not significant |
| Height | 2.7667 | 0.30962 | 2.7406 | 0.34486 | 0.724 | Not significant |
| Weight | 49.65 | 14.615 | 49.67 | 12.900 | 0.994 | Not significant |
| BMI | 18.1579 | 5.63828 | 18.3662 | 5.18084 | 0.853 | Not significant |
| Income | 20565.22 | 20362.234 | 15415.66 | 13794.854 | 0.096 | Not significant |
| Gaps(M) | 1.9500 | 0.76194 | 1.5000 | 0.78288 | 0.082 | Not significant |

## DISCUSSION

The current study was designed to estimate the frequency of drug resistance among tuberculosis relapses and factors contributing to it. Findings revealed that 6.5% patients were infected with MDR-TB, and various risk factors like gender, age, marital status, weight, BMI, duration of previous PTB treatment, drug side effects, and gaps in drug interruption were associated with the incidence of MDR-TB and PTB relapse. The increasing occurrence of MDR-TB is a significant threat to TB control programs in developing countries. The occurrence of RR-TB in this study population is a severe concern for the health authorities.

The drug interruption during first episode of TB was found to be the most significant factor responsible for relapse cases of TB and in our study MDR-TB was more prevalent in patients with longer gaps of drug interruption with mean gap of (1.9500±0.7694 p=0.082) months in previous PTB treatment as compared to shorter gaps of drug interruption, this is supported by results of a previous study in which total 15 (17.8%) cases of PTB relapse had completed 08 months while 6 (7.1%) of the cases were on ATT for 9 months13.

The younger age of the patients was associated with an increased risk of disease. This study also showed that MDR-TB is more prevalent in younger age groups as compared to older age groups and those with previous history of PTB treatment, as mean age for rifampicin resistance group was 36.17±16.615 (p=0.543) years, this finding is similar to as described in a previous study where child's age (AOR: 1.43; 95% CI, p = 0.002) and previous TB treatment history (AOR: 2.51; 95% CI, p = 0.01) were independent risk factors for infection, as children exposed to drug-susceptible TB, furthermore in this study patients of drug resistance had history of PTB treatment (6month=5.9%, 7months=1.9%, 8month=13.3%, 9months,11.1%,p=0.103)10.

Marital status was also associated with MDR-TB as it is more prevalent in married individuals as compared to unmarried( married=19(6.0)%, unmarried=4(11.1)%, p=0.273 ). Supported by another study done in Cameroon, in which married participants recorded more drug-resistant TB than unmarried participants. Most of the participants were male (51.2%) and fell within the age group of 31 to 45 years old (38.4%). Over fifty percent (55.5%) were married, a (Married=924 (55.5%) Single=741(44.5%)p = 0:006)11.

Reduced body weight and lower BMI are also stronger risk factors for both TB relapse and drug resistance. This study showed that MDR-TB is more prevalent in low-weight and lower BMI patients, i.e, Weight=49.65±14.615 P=0.994, BMI±18.15±5.638 P=0.853. Similar results were shown in another study in which patients having a baseline body mass index (BMI) less than 18.5Kg/m2, and having a history of pulmonary TB were determinants of MDR-TB. Therefore, priority should be given to malnutrition screening as a first-line diagnosis, nutritional supplements, and health education about proper housing12.

A study done by Hashmi et. al in 2017 shows slightly lower prevalence of MDR-TB in Pakistan(3.7%). .14Additionally, a higher prevalence rate of RR-TB was reported from other regions of the world, like Ethiopia in 2017(9.8%) 15. Particularly in Pakistan, inadequate diagnostic and therapeutic facilities in association with other socioeconomic crises play a significant role in the emergence of MDR-TB. A range of data has been reported from Pakistan showing an increasing incidence rate of MDR-TB as well as extensively drug-resistant TB (XDR-TB). It has been reported that the incidence rate of MDR-TB has jumped from 5% to almost 24%16.

Moreover, in the present study, we also assessed the

diagnostic yield and sensitivity of GeneXpert in identifying MDR-TB in sputum samples compared with microscopy, considered the gold standard in TB diagnosis. Several studies have reported the diagnostic efficacy of GeneXpert17.

The other studies from Pakistan have also reported the diagnostic accuracy of GeneXpert, which revealed that it is a significantly sensitive as well as specific tool for MDR-TB diagnosis as compared to other conventional diagnostic approaches18.
The gender factor was also associated with MDR-TB, in the current study MDR-TB is more prevalent in females as compared to males(7.5%>5.6%).this finding is same as described in a previous study(M: F=80:100) done in Eswatini, Netherlands, Namibia, Singapore and the USA, comparing gender as risk factors for MDR-TB19.

The results of the study carry substantial significance due to the limited data available regarding the occurrence of MDR-TB. Since antibiotic resistance is an evolving mechanism, there is a need for continuous monitoring of MDR-TB strains. Some limitations of the study include bias in patient selection since the study was based at a TB center, results of the study may not be helpful for the overall TB status in the region as samples were collected from pulmonary patients and a strict exclusion criterion was adopted for the collection of samples which may be differ from the actual field situation.

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