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# Molecular Characterization and RIF Resistance Mutation Pattern in Multi Drug Resistance Isolates Using Xpert MTB/RIF Assay in Clinical Isolates from Quetta, Pakistan

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**ABSTRACT**

Multidrug-resistant tuberculosis (MDR-TB) is currently one of the serious global challenges. With the advancement in the diagnostic facilities against tuberculosis and the advent of Xpert assay, the diagnosis of MDR-TB has become easier and more reliable. A total of 6353 specimens received at Fatima Jinnah Chest Hospital, Quetta, were screened through fluorescent microscopy and GeneXpert assay from 2016 to 2018. The data collected were analyzed using SPSS. Out of 6353 specimens, 1297 mycobacterium tuberculosis (MTB) positive cases were detected by Xpert assay, with 184 demonstrating rif resistance, mutations in the 81bp RRD region. The frequency of different probes was as follows: probe E 98/184 (53%), D 23/184 (12.5%), B 15/184 (8.1%), C 5/184 (2.7%), A 3/184 (1.6%), double probe mutation 5/184 (2.7%), D&E 3/184 (1.6%), and C&D 2/184 (1%) and all 5-probe mutation 35/184 (19%). A statistically significant association was found between probe type and gender and treatment history, while no significant association was found between probe type and age group of patients. MDR-TB prevalence was 184/1297 (14.18%) with dominance of Probe E (53.26%) in the population studied. This study explores the increasing pattern of MDR-TB in the area. Effective measures should be adopted to curtail the disease in the area.



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## 1. Introduction

The emergence of multidrug-resistant tuberculosis (MDR-TB) poses a serious threat throughout the globe. The situation worsens when fluoroquinolones show resistance during treatment against tuberculosis (Ahmad et al., 2016).

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There are about 22 high-ranked TB burden countries globally, and Pakistan ranks 5th among them. Even worse, it ranks 4th among countries with MDR-TB. Tuberculosis is more likely to be transmitted from infected patients to healthy individuals in close vicinity, leading to MDR-TB. In recent years, tuberculosis diagnosis has been revolutionized, especially, with the addition of Xpert assay, that can also detect rif resistance from isolates (Boehme et al., 2010). Largely, resistance of Mycobacterium strains against rifampicin is caused by rpoB gene mutations globally, with 96.1% occurring at amino acid residues 507-533, in the Rifampicin resistance determining region (RRDR) (Ramaswamy et al., 1998). In Pakistan, the frequency of MDR TB in new and previously treated patients was 4.2% and 16%, respectively (WHO, 2018). Most of the MDR TB cases are due to mutations in the promoter or gene region that encodes the drug target (Ong et al., 2010). Generally, M tb isolates show resistance to one or multiple drugs, such as Rifampicin (RIF), Isoniazid (INH), Ethambutol (EMB), and Streptomycin (STR), which further complicates its treatment. Different studies have shown that RIF resistance is due to mutation in rpoB, while resistance in INH is due to KatG and ahpC (Ramaswamy et al., 1998), and in EMB due to embB (Telenti et al., 1997) and pncA (Scorpio et al., 1996), Early and rapid detection of RIF resistance is of utmost necessity in the effective control of MDR TB, as it is one of the surrogate markers of MDR resistance (Heep et al., 2001). Studies have shown that about 98% of RIF resistant isolates have mutations in the rpoB gene encoding the beta subunit of RNA polymerase. Limited data are documented on mutations in the rpoB gene from Balochistan, Pakistan. Hence, this study was aimed to establish regional reference line data on various mutations in different rpoB genes in RRD Region using Xpert assay.

## 2. Methodology

### *2.1. Study design and setting*

This retrospective cross-sectional study was carried out at Fatima Jinnah TB Hospital, Brewery Road, Quetta, Pakistan. Pulmonary and extrapulmonary samples were taken from outpatient, and indoor patients during 2016–18. Samples were first screened through fluorescent microscopy, and the positive samples were again subjected to Xpert assay for confirmation of tuberculosis and estimation of rif resistance.

### *2.2. Ethical Statement*

This study was approved by the research and ethics board of the authorities of Fatima Jinnah Chest and General Hospital, Quetta. Written and verbal consents were taken prior to the study. In the case of illiterate subjects, verbal consent was explained in the native language.

### *2.3. Clinical Specimen*

A total of 6353 specimens were subjected to GeneXpert for the detection of Mycobacterium with rifampicin resistance. Similarly, frequencies of different probes were also estimated. The frequencies of different probes in Mycobacterium tuberculosis (MTB) cases are shown in Figure 1.

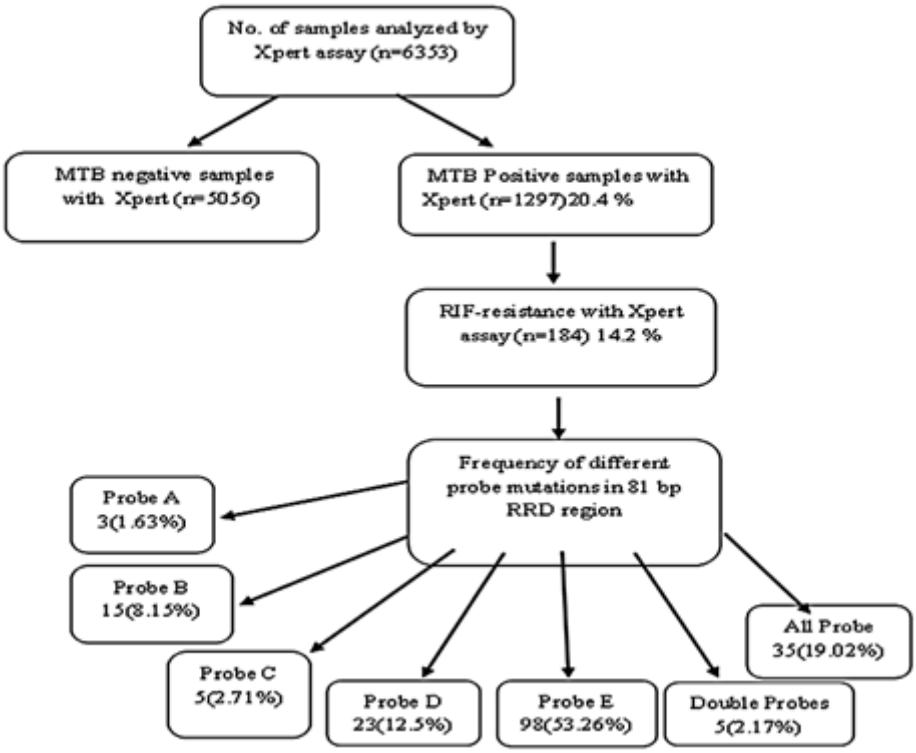


**Figure 1.** Frequencies and percentages of different probes in MTB cases

*2.4. Criteria for Xpert Analysis*

All fluorescent microscopy (FM) smear positive samples from outpatients were considered. The specimens from indoor patients were sputa, nasopharyngeal aspirates, gastric aspirates, pleural fluid and cerebrospinal fluid

*2.5. MTB Rif Assay*

Smears were prepared with Auramine-Rhodamine dye using FM staining. The collected specimens were decontaminated using the N-acetyl-L cysteine-NaOH technique, (Javaid et al., 2008). This solution was added to the sample at a (1:2) ratio, followed by vortexing and centrifugation at 1000 RPM for 5 minutes. About a 2-3 ml sample was transferred into the cartridge and loaded onto the Xpert according to the manufacturer's guide.

*2.6. Statistical Analysis*

The data collected were analyzed using SPSS software version 20. A statistically significant association was found between probe type and gender and treatment history, while non-significant between probe type and age group of patients using Pearson Chi square test

## 3. Results and Discussions

In this study, more MDR cases with Rif resistance were recorded: 116 (63%) in female patients and 68 (37%) in male patients. A higher number of MDR cases were in newly diagnosed patients 105/184 (57%) whereas 79, (43%) cases were in previously treated patients. Similarly, all the probes were detected in patients aged between 10 and 70 years, and the fewest MDR cases were noted in patients aged below 10 or above 70. Out of 1297 MTB positive cases, 184

(14.2%) were resistant to Rifampicin using Xpert® MTB/RIF assay. This resistance was due to a mutation in five dissimilar rpoB genes present in the 81 bp RRDR of the mycobacteria genome. The mutation frequency in these five different rpoB genes detected were as follows: Probe E (Codon, 531), Probe B (Codon, 513), D (Codon, 526), A (Codon, 511) and C (Codon, 522). Their frequency distribution was Probe E 98/184 (53%), B 15/184 (8.1%), D 23/184 (12.5%), A 3/184 (1.6%) and C 5/184 (2.7%). Similarly, double probe mutations were in D & E, 3/184 (1.6%) and C & D 2/184 (1%), and all 5 probe mutations were 35/184 (19%), respectively.

**Table 1.** Frequency of probes for various rpoB gene mutations in the 81 bp RRDR of rpoB gene in rifampicin resistance detected (RRD) cases of Mycobacterium tuberculosis with different demographic representation.

| **Demography** | **Probe types** | | | | | | | | Total (%) |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | D & E | C & D | All | |
| Frequency (%) | 3 (1.6) | 15 (8.1) | 5 (2.7) | 23 (12.5) | 98 (53.4) | 3 (1.6) | 2 (1.0) | 35 (19.0) | 184 (100) |
| **Gender** | | | | | | | | | |
| Female | 2 | 9 | 3 | 17 | 69 | 0 | 0 | 16 | 116 (63) |
| Male | 1 | 6 | 2 | 6 | 29 | 3 | 2 | 19 | 68 (37) |
| **History** | | | | | | | | | |
| New patients | 1 | 7 | 4 | 8 | 72 | 2 | 2 | 9 | 105 (57) |
| Previously treated | 2 | 8 | 1 | 15 | 26 | 1 | 0 | 26 | 79 (43) |
| **Age categories** (Year) | | | | | | | | | |
| <10 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 5 (2.7) |
| 10-20 | 1 | 3 | 2 | 5 | 9 | 1 | 0 | 2 | 23 (12.5) |
| 21-30 | 0 | 2 | 1 | 7 | 23 | 0 | 0 | 12 | 45 (24.4) |
| 31-40 | 0 | 1 | 0 | 4 | 9 | 0 | 0 | 4 | 18 (10) |
| 41-50 | 1 | 5 | 1 | 5 | 18 | 1 | 1 | 9 | 41 (22) |
| 51-60 | 1 | 2 | 1 | 0 | 19 | 1 | 1 | 5 | 30 (16) |
| 60-70 | 0 | 1 | 0 | 1 | 15 | 0 | 0 | 1 | 18 (9.7) |
| >70 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 4 (2) |

The genetic basis of drug resistance against MTB involves a type of mutation known as point mutation in some of the most significant genes such as katG, rpoB, embB, and rpsL, etc. (Ramaswamy et al., 1998). MDR-TB is one of the biggest threats to human life worldwide, particularly to individuals living in industrialized or technologically advanced countries. We used the Xpert assay in our study to detect mycobacterium resistance to rifampicin due to the mutation in the rpoB gene responsible for RIF resistance. It is accompanied by isoniazid resistance in 90% of cases of MDR-TB (Ioerger et al., 2009). Balochistan is the largest province of Pakistan with over two million people. It also shares the major portion of its border with the war-stricken Afghanistan and Iran. This study is significant as there is limited or no data available on MDR TB in Balochistan Province. This study will provide baseline data for MDR TB with rifampicin resistance in Balochistan.

The highest frequency of mutation in the 81 bp RRD region was with probe E (53.26%), followed by D (12.5%) and B (8.15%). While the least frequency was observed in Probe A (1.6%) and C (2.7%), further analysis is needed to determine its significance. These findings corroborate with studies from Quetta, Pakistan (Siddique et al., 2019), China (Yue et al., 2003), Khyber Pakhtunkhwa, Pakistan (Ullah et al., 2016), Punjab, Pakistan (Khan et al., 2013), and Uganda (Mboowa et al., 2014), which found that probes E and B were the most common sites of mutation in the RRD region for Rif resistance in clinical samples using the Xpert assay. The probe C was uncommon and rarely

found in the world, as reported in a study from Zimbabwe (Metcalfe et al., 2016). We have also reported mutations in double probes, specifically D&E (1.6304%) and C&D (1.0869%), with a total occurrence of 2.7173%, and mutations in all 5 probes (19.0217%). Other researchers (Reddy et al., 2017, and Ullah et al., 2016) also support our findings on the detection of multiprobes in this region.

Overall, 184/1297 out of 1297 cases (14.18 %) resistant to rifampicin were recorded in clinical isolates of Quetta. Similarly, in China, 30.4% were reported (Liu et al., 2013), in India 9.2% (Reddy et al., 2017), and in Brazil 44.3% (Luiz et al., 2013). Rif resistance has been recorded in other studies from around the world, while in previous studies from Pakistan, 11.3% and 11.5% resistance have been reported (Ullah et al., 2016). In another case from Karachi, a much lower 5% of MDR TB cases were recorded in 2010 (Ejaz et al., 2010). This higher MDR rate in the same country might be due to improved and accurate diagnosis through Xpert assay. In this study, 105 (57%) MDR cases with rifampicin resistance were from newly diagnosed patients as compared to 79 (43%) previously treated cases. This contradicts a previous report from Pakistan that 4.3% of MDR with Rif resistance cases were new, while 19.4% of previously treated cases were identified (WHO, 2018). This variance might be a result of different geographical location, or favorable dry windy weather, and ample sample size. Resistant tuberculosis was equally identified in almost all age groups preferably among the most productive age group (20-60 years) (Table 1). Multidrug resistance is the new challenge in health care centers globally for almost all infectious diseases, but tuberculosis poses the most serious threat while treating it.

To achieve sustainable development goals (SDGs), WHO approved and recommended the adoption of Xpert assay in the diagnosis of tuberculosis, especially in countries with a high burden of MDR-TB. It should be noted that Xpert assay is highly valuable for the early detection of MDR TB, especially in rural settings of Balochistan province.

## 4. Conclusions

MDR-TB prevalence was 184/1297 (14.18 %) with Probe E (53.26 %) dominance in the studied population. This study explores the increasing pattern of MDR-TB and paves the road for similar studies to be conducted in the area. Concrete and strong measures should be adopted to curtail the disease in the area.

## Abbreviations

RIF, rifampicin/rifampin; RRD-Region, rifampicin resistance determining region; rpoB, RNA Polymerase Beta subunit; RRD cases, rifampicin resistance detected cases.

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## Declaration of Competing Interest and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in OPS Journal belongs to the authors.

## References

Ahmad, N., Javaid, A., Sulaiman, S. A., Ming, L. C., Ahmad, I., & Khan, A. H. (2016). Resistance patterns, prevalence, and predictors of fluoroquinolones resistance in multidrug resistant tuberculosis patients. *The Brazilian Journal of Infectious Diseases, 20*(1), 41-47. https://doi.org/10.1016/j.bjid.2015.09.011

Boehme, C. C., Nabeta, P., Hillemann, D., Nicol, M. P., Shenai, S., Krapp, F., Allen, J., Tahirli, R., Blakemore, R., Rustomjee, R., Milovic, A., Jones, M., O'Brien, S. M., Persing, D. H., Ruesch-Gerdes, S., Gotuzzo, E., Rodrigues, C., Alland, D., & Perkins, M. D. (2010). Rapid molecular detection of tuberculosis and rifampin resistance. *The New England Journal of Medicine, 363*(11), 1005–1015. https://doi.org/10.1056/NEJMoa0907847

Ejaz, M., Siddiqui, A. R., Rafiq, Y., Malik, F., Channa, A., Mangi, R., Habib, F., & Hasan, R. (2010). Prevalence of multi-drug resistant tuberculosis in Karachi, Pakistan: Identification of at risk groups. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 104*(8), 511-517. https://doi.org/10.1016/j.trstmh.2010.03.005

Heep, M., Brandstätter, B., Rieger, U., Lehn, N., Richter, E., Rüsch-Gerdes, S., & Niemann, S. (2001). Frequency of rpoB mutations inside and outside the cluster I region in rifampin-resistant clinical Mycobacterium tuberculosis isolates. *Journal of Clinical Microbiology, 39*(1), 107-110. https://doi.org/10.1128/JCM.39.1.107-110.2001

Ioerger, T. R., Koo, S., No, E. G., Chen, X., Larsen, M. H., Jacobs Jr, W. R., Pillay, M., Sturm, A. W. & Sacchettini, J. C. (2009). Genome analysis of multi-and extensively-drug-resistant tuberculosis from KwaZulu-Natal, South Africa. *PLoS ONE, 4*(11), e7778. https://doi.org/10.1371/journal.pone.0007778

Javaid, A., Hasan, R., Zafar, A., Ghafoor, A., Pathan, A. J., Rab, A., ... & Shaheen, Z. (2008). Prevalence of primary multidrug resistance to anti-tuberculosis drugs in Pakistan. *The International Journal of Tuberculosis and Lung Disease, 12*(3), 326-331.

Khan, S. N., Niemann, S., Gulfraz, M., Qayyum, M., Siddiqi, S., Mirza, Z. S., Tahsin, S., Ebrahimi-Rad, M., & Khanum, A. (2013). Molecular characterization of multidrug-resistant isolates of Mycobacterium tuberculosis from patients in Punjab, Pakistan. *Pakistan Journal of Zoology, 45*(1), 93–100.

Liu, Q., Zhu, L., Shao, Y., Song, H., Li, G., Zhou, Y., Shi, J., Zhong, C., Chen, C., & Wei, L. (2013). Rates and risk factors for drug resistance tuberculosis in Northeastern China. *BMC Public Health, 13*(1), 1171. https://doi.org/10.1186/1471-2458-13-1171

Luiz, R. D. S. S., Suffys, P., Barroso, E. C., Kerr, L. R. F. S., Duarte, C. R., Freitas, M. V. C., Mota, R. M. S., & Frota, C. C. (2013). Genotyping and drug resistance patterns of Mycobacterium tuberculosis strains observed in a tuberculosis high-burden municipality in Northeast, Brazil. *The Brazilian Journal of Infectious Diseases, 17*(3), 338-345. https://doi.org/10.1016/j.bjid.2012.10.019

Mboowa, G., Namaganda, C., & Ssengooba, W. (2014). Rifampicin resistance mutations in the 81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert® MTB/RIF in Kampala, Uganda: A retrospective study. *BMC Infectious Diseases, 14*(1), 481. https://doi.org/10.1186/1471-2334-14-481

Metcalfe, J. Z., Makumbirofa, S., Makamure, B., Sandy, C., Bara, W., Mason, P., & Hopewell, P. C. (2016). Xpert® MTB/RIF detection of rifampin resistance and time to treatment initiation in Harare, Zimbabwe. *The International Journal of Tuberculosis and Lung Disease, 20*(7), 882-889. https://doi.org/10.5588/ijtld.15.0696

Ong, D. C., Yam, W. C., Siu, G. K., & Lee, A. S. (2010). Rapid detection of rifampicin-and isoniazid-resistant Mycobacterium tuberculosis by high-resolution melting analysis. *Journal of Clinical Microbiology, 48*(4), 1047-1054. https://doi.org/10.1128/jcm.02036-09

Ramaswamy, S., & Musser, J. M. (1998). Molecular genetic basis of antimicrobial agent resistance inMycobacterium tuberculosis: 1998 update. *Tubercle and Lung disease, 79*(1), 3-29. https://doi.org/10.1054/tuld.1998.0002

Reddy, R., & Alvarez-Uria, G. (2017). Molecular epidemiology of rifampicin resistance in Mycobacterium tuberculosis using the GeneXpert MTB/RIF assay from a rural setting in India. *Journal of Pathogens, 2017*, 6738095. https://doi.org/10.1155/2017/6738095

Scorpio, A., & Zhang, Y. (1996). Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nature medicine, 2*(6), 662-667. https://doi.org/10.1038/nm0696-662

Siddique, G., Shafee, M., Abbas, F., Naeem, M., Raziq, A., Ahmad, S., & Babar, K. M. (2019). Mycobacterium tuberculosis; frequency of rifampin resistance mutations in 81-bp rrdr of rpob gene in mycobacterium tuberculosis isolates in Quetta, Pakistan. *The Professional Medical Journal, 26*(05), 780-786. https://doi.org/10.29309/TPMJ/2019.26.05.3477

Telenti, A., Philipp, W. J., Sreevatsan, S., Bernasconi, C., Stockbauer, K. E., Wieles, B., Musser, J. M. & Jacobs Jr, W. R. (1997). The emb operon, a gene cluster of Mycobacterium tuberculosis involved in resistance to ethambutol. *Nature Medicine, 3*(5), 567-570. https://doi.org/10.1038/nm0597-567

Ullah, I., Javaid, A., Tahir, Z., Ullah, O., Shah, A. A., Hasan, F., & Ayub, N. (2016). Pattern of drug resistance and risk factors associated with development of drug resistant Mycobacterium tuberculosis in Pakistan. *PLoS ONE, 11*(1), e0147529. https://doi.org/10.1371/journal.pone.0147529

Ullah, I., Shah, A. A., Basit, A., Ali, M., Khan, A., Ullah, U., Ihtesham, M., Mehreen, S., Mughal, A., & Javaid, A. (2016). Rifampicin resistance mutations in the 81 bp RRDR of rpo B gene in Mycobacterium tuberculosis clinical isolates using Xpert MTB/RIF in Khyber Pakhtunkhwa, Pakistan: A retrospective study. *BMC Infectious Diseases, 16*(1), 413. https://doi.org/10.1186/s12879-016-1745-2

World Health Organization -WHO- (2018). *Global tuberculosis report 2018*. Geneva, Switzerland: World Health Organization, WHO Press.

Yue, J., Shi, W., Xie, J., Li, Y., Zeng, E., & Wang, H. (2003). Mutations in the rpoB gene of multidrug-resistant Mycobacterium tuberculosis isolates from China. *Journal of Clinical Microbiology, 41*(5), 2209-2212. https://doi.org/10.1128/jcm.41.5.2209-2212.2003