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Detection and Drug Resistance Analysis of *Mycobacterium tuberculosis* Using GeneXpert in Mardan, KPK

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ABSTRACT

Background: Tuberculosis is one of the most widespread infectious diseases in the world contributing to high morbidity and mortality. Pakistan ranks 5th among the countries with the highest tuberculosis (TB) burden and 6th among those with the highest drug-resistant TB including multi-drug resistant (MDR-TB) and extensively drug resistant (XDR-TB). The present study aims to evaluate GeneXpert-based detection and drug resistance analysis of *Mycobacterium tuberculosis* in a tertiary care hospital of Mardan Khyber Pakhtunkhwa.

Method: A cross-sectional study was conducted to collect 96 sputum samples from pulmonary TB patients between 1st January to 30th April 2025. The sample were analyzed using GeneXpert following the standard protocol. Statistical analysis was performed using SPSS version 25.0, and Chi-square tests were applied to assess associations, with a p-value < 0.05 considered significant.

Results: Out of 96 samples, 69 (71.9%) were tested positive for *Mycobacterium tuberculosis*. Among the positive cases 67 % were identified as multi-drug-resistant TB (MDR-TB) while 33% were extensively drug-resistant TB (XDR-TB). The Gene Xpert (PCR) demonstrated different sensitivity in detecting drug resistance of 29% for fluroquinolone, 6.0% for ethambutol, 3.0% for kanamycin, 3.0 % for Amikacin and 60% for Isoniazid. The Chi-square test revealed a significant association between drug resistance patterns and TB types ($\chi^2 = 15.8$, $p = 0.003$).

Conclusion: The high prevalence of drug-resistant TB in Pakistan highlights the urgent need for continuous surveillance and universal drug susceptibility testing. These findings highlight the ongoing challenge of drug-resistant TB in the region and emphasize the importance of rapid molecular diagnostic tools like GeneXpert for early detection and management.

Keywords: Tuberculosis, GeneXpert, Multi drug resistant (MDR-TB), Extensively drug-resistant TB (XDR-TB), Mardan, Khyber Pakhtunkhwa (KPK).

INTRODUCTION

Mycobacterium tuberculosis (MTB) is anaerobic bacteria which is spread through airborne droplet and can cause pulmonary and extra pulmonary tuberculosis (TB). Mycobacterium TB is an obligatory aerobe, it infects tissues with high oxygen content, including infection of lungs, meninges, Kidney, bone and soft tissue.[1] The causative agent of tuberculosis is *Mycobacterium tuberculosis* (MTB), which is considered to be the leading cause of this disease. Additionally, the cell wall of these bacteria contains special mycolic acids that are essential to the wall's structure and functionality. TB is caused by the *Mycobacterium tuberculosis* complex, which is described as the causative agent of TB in differenthosts including *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, and *Mycobacterium microti*, while *Mycobacterium caprae* and *Mycobacterium pinnipedii* as variants of *Mycobacterium bovis*.[2,3]

According to World Health Organization (WHO), 1.4 million people died with tuberculosis (TB) in 2019, and about ten million others had the disease. According to estimates, 10.6 million people got tuberculosis in 2021, up from 10.1 million in 2020, and 1·6 million people died from the disease in 2021.[4] In 2021, there were

450,000 incident instances of rifampicin-resistant tuberculosis, indicating a 3% rise in the rate of drug-resistant tuberculosis between 2020 and 2021. Additionally, it was stated that approximately 1.3 million people died with tuberculosis yearly. According to the WHO Report in 2020, India, China, Indonesia, Philippines, Pakistan and Bangladesh are six of the eight TB-affected nations in Asia that account for two-thirds of all TB cases worldwide.[5]

There are in fact three different types of tuberculosis: extensively drug-resistant (XDR), pre-extensively drug-resistant (Pre-XDR) and multi-drug-resistant (MDR). The expected number of MDR event cases in 2021 was 450,000, up 3.1% from 437,000 in 2020. In 2021, the Russian Federation (8.5%), India (26%), and Pakistan (7.9%) accounted for 42% of all cases globally.[6] In 2021, WHO approved the pre-XDR-TB classification (MDR-TB+floroquinolone resistance) and modified the definition of XDR-TB to include MDR-TB+further resistance to any floroquinolone and at least one WHO Group A drug (bedaquiline, linezolid).[7]

Various diagnostic techniques are used for the examination of tuberculosis. The diagnosis of TB is rarely confirmed or suggested by routine laboratory testing. Due to its affordability in situations with limited resources, microscopic examination for AFB remains relevant even in the current era of molecular diagnostics.[8] Successful patient outcomes and early patient care depend on early diagnosis. Since Ziehl-Neelsen (ZN) AFB smear microscopy, which is used in the majority of TB control programs, has low sensitivity and necessitates numerous visits, which raises the default rate, false-negative findings and misinterpretation of TB suspects are prevalent in developing countries.[9] WHO-recommended GeneXpert MTB/RIF assay is a complete automated cartridge-based molecular system that transforms the world of tuberculosis (TB) control as it participates in novel ways to control the tuberculosis disease along with drug resistance in patients whose disease might go unnoticed through conventional screening tests.[10] There is a proxy relationship between Rifampicin-resistant tuberculosis (RR-TB) and MDR-TB with over 90 percent of cases.[11] GeneXpert MTB / RIF testing is a molecular diagnostic test used to run less than two hours, using automated cartridge based GeneXpert machine (Cepheid, Sunnyvale, CA, USA).[12]

This study aims to detect and analyze drug resistant *Mycobacterium tuberculosis* in pulmonary TB patients by using GeneXpert, and also evaluate the sensitivity of GeneXpert in identifying resistance to first-line and second-line anti-tuberculosis drugs in a tertiary care hospital of Mardan, Khyber Pakhtunkhwa.

METHODOLOGY

This cross-sectional study was carried out at the Mardan Medical Complex (MMC) TB Section, Mardan, Khyber Pakhtunkhwa, Pakistan. The Research study was approved by authorities of MMC hospital. Written informed consent was taken from all participants after being briefed on the study's objectives, procedures, and their right to withdraw. Total 96 sputum samples were collected from pulmonary TB patients a tertiary healthcare facility in Mardan, Khyber Pakhtunkhwa, Pakistan, from 1st January 2025 to 30th April 2025. The sample size was calculated according to WHO guidelines by the formula $n = (Z^2 P(1-P)) / d$.[13]

Standard protocols were followed for sample preparation and analysis. Sputum samples were taken from patients of various ages, both male and female. Every patient had their deep cough sputum collected in the early morning; each specimen had a minimum volume of two milliliters. The Xpert MTB/RIF assay was utilized to diagnose tuberculosis from a single sputum sample per patient. In short, the sputum was collected and then combined with sample reagent at a volume ratio of 1:2 (sample: sample reagent). Sputum can be thick and viscous making it difficult to mix and pipette accurately. The sample reagent helps reduce the viscosity and break down the mucous components of the sputum, creating a more homogeneous sample. The bacteria in the sample is inactivated by sample reagent. After 15-20 mins the sample was consistent and was ready for PCR reaction. Xpert MTB/RIF cartridge was labeled with laboratory number on the side of cartridge. A processed sample of 2 ml were added to the cartridge using the Pasteur pipette. The cartridge's top was opened, and the sample was carefully poured slowly to reduce the possibility of aerosol generation. Place the sample that has been processed with the sample reagent into the Xpert MTB/RIF cartridge's sample chamber. Properly close the cartridge lid [14]. Finally, the GeneXpert MTB/RIF assay machine was loaded with the cartridge containing the specimen. The sample completed concentration, amplification and detection of the rpoB DNA segment in all following processing steps which were entirely automated. The GeneXpert® Dx System showed the results after one hour and twenty-five minutes. The used cartridge was discarded immediately. For this, nitrile gloves, safety goggles, PPE kits and a procedure mask were utilized. According to the evaluation form, Results were considered positive if M. tuberculosis was detected with or without Rifampicin resistance and negative if no MTB was detected.[15]

Match the patient identification number with the request slip. Identification number on the sputum container must be co relate with cartridge. Sampling identification is very crucial step for test performance. It must be remember that gene Xpert assay is properly functioning and the current model for the test is available. Turn on the system with gene Xpert machine, Run the GeneXpert Dx icon in the system with a personal log in ID, and password. Click on "Check Status" and check if modules are available, if not, proceed to "Troubleshooting" in Gene Xpert Binder.

Tests are performed and results reported as specified in manufacturer package insert without substitution of reagents. The PPE are also maintained. Wear gloves and disinfect the working area where you are performing the test. Label one 15 mL conical with specimen identification number for each specimen separately. Aliquot 1 mL specimen in 15 mL conical container and added 2 mL of Sample Reagent in 2:1 v/v ratio, then close the cap and vortex well for at least 10 seconds. Incubate at room temperature for a total of 15 minutes and finally vortex for 10 seconds. It must be noted that Samples should be liquefied with no visible clumps of sputum. If there are still clumps of sputum, vortex again and incubate for another 10-15 min.

Descriptive analysis of data was used to summarize the demographic and clinical characteristics of the study population. The patterns of drug resistance were placed in categories and presented in the form of frequencies, percentages, and 95% Confidence Intervals (95% CI) with the case of MDR-TB and XDR-TB. GeneXpert sensitivity in regard to first-line anti-TB drug resistance and second-line anti-TB drug resistance was determined and reported in tables and graphs. The data were interpreted within the frames of the literature whereby the accuracy was checked through statistical software (SPSS version 25.0) and the trends along with implications analyzed. Chi square test were applied for statistical analysis and comparison, p-value (0.005) was considered significant.

RESULTS

Total 96 samples were collected and processed, the assay provided valid results in (n=88) 91.67% samples and unsuccessful results in (n=8) 8.33% samples because of un-consistent samples (Table 1). Hence, the total numbers of samples eligible in the research were 88. Majority of the samples were male 52.27% compared to female 47.72%. Anatomically, most samples were from pulmonary TB (PTB) cases 77.6% and newly diagnosed TB cases accounted for the majority in the treatment history group 56.3%.

Table 1. GeneXpert MTB/RIF Assay Results and Patient Demographics.

TB Data	Patients No
Total Cases	96
Error	8
TB Detected	69
Non-Detected	19
Male	46
Female	42

The prevalence rate of XDR *Mycobacterium tuberculosis* during the study period was (78%) 69 out of 88 (95% CI: 68.9% - 86.7%) among TB patients which were screened on Gene Xpert while non-detected TB patients were 22% (95% CI: 54.6% - 77.9%) as shown in the Figure 1.

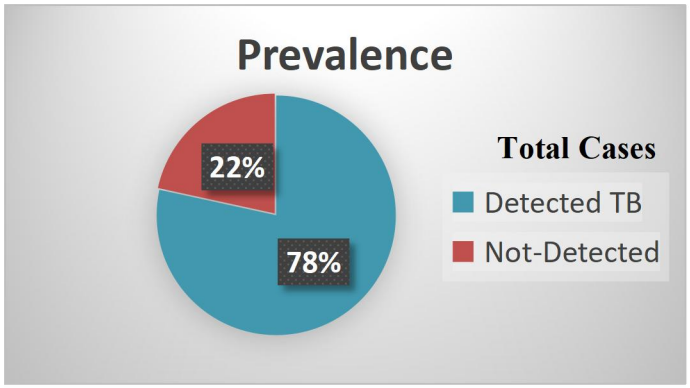

Figure 1. Pie chart shows the prevalence rate of TB patients in MMC.

Antibiotic synergy is used for the treatment of tuberculosis. There are two types of drugs phases, (Intensive) first and second (Continuation) line of drugs. Isoniazid, pyrazinamide, ethambutol and rifampicin are the first line of drugs (Intensive Phase) used for first two months. In our research, Isoniazid shows 61% Resistance and Ethambutol shows 6% resistance which is considered MDR infections in Mardan Medical complex. In overall study, it is concluded that 67% are MDR infections while 33% are XDR infections. Floroquinolone (Levofloxacin and Moxifloxicin) and Injectable (Amikacin, kanamycin, Capromycin) are the second line drugs that shows 33% resistance in the current study is shown in the Figure 2 and 3 respectively. The Chi-square test showed a significant association between drug resistance type and TB detection ($\chi^2 = 15.8$, $p = 0.003$).

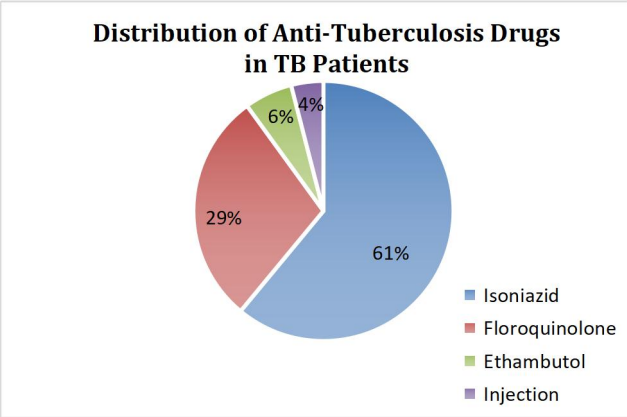

Figure 2. 1st and 2nd line of TB Drugs used for the treatment

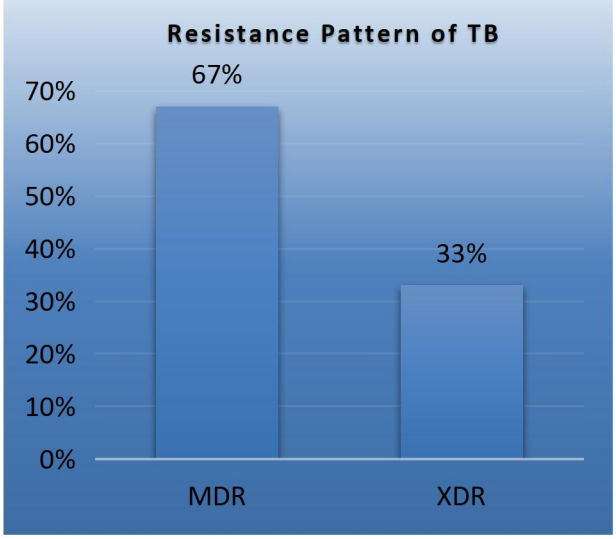

Figure 3. Resistance profile of *Mycobacterium tuberculosis* (MDR and XDR).

DISCUSSION

This study was carried out to address the prevalence of *Mycobacterium tuberculosis* at Mardan Medical Complex Hospital by using the Gene Xpert diagnostic technique. It also aimed to provide useful information concerning the risk factors that have been associated to the disease. We have identified several important clinical and related factors of tuberculosis for the Society and have obtained a result from the study that shows tuberculosis is a public health concern. As a chronic infectious illness, tuberculosis (TB) is a significant global public health concern and continues to be a major obstacle for poor nations. Latent tuberculosis, which is brought on by an infection with *Mycobacterium tuberculosis*, affects over one-third of the global population. Drug-resistant TB exacerbates the illness, and quick, extremely sensitive diagnostic techniques are desperately needed. A new real-time polymerase chain reaction test called Gene Xpert (GX) can identify *Mycobacterium tuberculosis* (MTB) and rifampin resistance (RIF) at the same time. TB infections are treated with first and second lines of therapy.

Studies reported an 8% prevalence rate and 18.98% prevalence rate of TB in 2022 in Pakistan, Columbia (41%), Dakar (28.6%), Canada (50%), Algeria (34.37%) and Ghana (44%), all reported prevalence rates that were roughly the same.[16,17] In comparison to the previous studies, which revealed 6.5% and 7.9% in Ethiopia, 13.8% in Nepal, and 12% in India, the incidence rate of M. tuberculosis (78%) in this study was higher.[18]

In our study, 52.27% were male patients and 47.72 were female which were diagnosed as TB patients and have a high resistance pattern of MTB drugs, while another study reported 60% male and 40% female in his study. Along with this they reported 51% illiterate and 58% rural area residents but in our study 63% secondary education people and 37% white color people were described.[16] Furthermore, 67% of MDR were reported in our study along with 33% of XDR which is a great threat for society and alarming condition, while a study reported 29.4% of MDR and 57% of XDR during nine years. 10.3% of resistance to first line of drugs and 16.2% of resistance to floroquinolone and other injections while in our study 60% resistance occurs to isoniazid and 29% resistance occur to floroquinolone. In general, 78% of tuberculosis cases were detected by Gene Xpert.[19] The trend of MTB and RR-MTB over time has also been attempted to be seen in this study. Therefore, a notable decline in the percentage of MTB frequency accompanied with a rise in the actual number of MTB detections indicates that the regional government and stakeholders must effectively address tuberculosis in the area. The absolute number of cases that tested positive for RR-MTB increased between 2016 and 2018, while this increase was not statistically significant.[20]

The Gene Xpert assay in this study demonstrated a sensitivity and specificity of 48% for INH resistance and 5% for both RMP and multidrug resistance. According to other findings, the GenoType MTB-DR plus assay's sensitivity and specificity performance nearly matches that of traditional culture-based susceptibility testing. A meta-analysis report revealed that the GenoType MTB-DR plus assay had a sensitivity and specificity of 96% and 100% for INH and 99% and 99% for RMP, respectively but another srudy reported a sensitivity of 95% for INH and 100% for RMP.[21,22] Another study reported 52.3% of

isoniazid resistance and 5.3% resistance to floroquinolone while our study shows 60% and 29% resistance to isoniazid and floroquinolone.[23]

This research has found high level of drug resistant TB in Mardan with 67 percent of the cases being MDR-TB and 33 percent XDR-TB. The Chi-square test served to validate the dependence between the patterns of drug resistance and TB types ($\chi^2 = 15.8$, $p = 0.003$), which reveals the intensity of the resistance profile in the area. Those results are consistent with the national and international reports on the increasing tendency of TB drug-resistance. Same study was conducted in Pakistan found that most isolates belonged to lineage 3 (n = 397; 74.2%) strain-types, and were MDR (n = 328; 61.3%) and (pre-)XDR (n = 113; 21.1%).[24]

This study identifies the alarming rate of drug-resistant TB in Mardan and suffers in terms of the low amount of participants, the clinic-based set up, and the statistical confirmation of them. The burden of TB in Pakistan should be reduced through increasing the use of GeneXpert testing, requiring adherence of treatment through DOT and limiting the overuse of antibiotics. It requires campaigns to address cough hygiene and get tested, and better infection control in hospitals. The elimination of resistance will involve universal DST pretreatment, new drugs on surveillance such as bedaquiline. There has to be a coherent message to the population that emphasizes testing, treatment adherence, and ventilation and supports policy changes and financing. TB is a disease that needs a coordinated response that is focused on diagnostics, stringent treatment guidelines, and community involvement.

CONCLUSION

This study revealed a high burden of drug-resistant tuberculosis, with 67% MDR-TB and 33% XDR-TB among confirmed cases. The alarming rate of fluoroquinolone-resistant MDR-TB highlights the critical need for improved drug regulation and treatment strategies. The high incidence of fluoroquinolone-resistant MDR-TB cases observed poses a serious public health concern. The growing burden of XDR-TB in Pakistan underscores the urgent need for strengthened TB control strategies Future studies should involve multiple centers across various districts with larger and more diverse sample sizes. Design follow-up or cohort studies to evaluate treatment outcomes, resistance development over time, and relapse rates.

Limitations of the study included: its design is single-center, hospital-based, which could limit external validity of the results since it could be subjected to referral bias; the use of the GeneXpert assay, but no routine culture confirmation of all samples, precludes gold-standard validation; the sample is relatively small, although it was calculated; cross-sectional design does not allow drawing causal inferences; and absence of detailed clinical and epidemiological information (e.g., a detailed treatment history) does not allow further study of resistance drivers. Nevertheless, these limitations do not negate the fact that this work presents the original molecular support of the high burden of MDR- and pre-XDR-TB in Mardan and, therefore, the necessity of a stricter surveillance.

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