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INCIDENCE OF FLUOROQUINOLONE AND AMINOGLYCOSIDE RESISTANCE VIA ONLINE PROBE ASSAY IN MULTIDRUG-RESISTANT TUBERCULOSIS CASES

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Abstract

Introduction: MDR-TB is a serious public health issue in places like Pakistan that report high cases. Anti-TB treatments are made harder, and XDR-TB becomes more likely when bacteria resist drugs other than the first-line drugs.

Objective: To know how often fluoroquinolone and aminoglycoside resistance occurs by using the Line Probe Assay in cases with MDR-TB.

Materials and Methods: The study was carried out at Mayo Hospital, Lahore, From September, 2024 to February, 2025. A Line Probe Assay was used on sputum from 150 MDR-TB patients to check for mutations making the infection resistant to both FQs and AGs.

Results: Of the total cases, 42% showed FQ resistance, 19.3% showed AG resistance, and 12% showed Pre-XDR (a combination of FQ and AG resistance). The gyrA and rrs genes were observed to have the most mutations.

Conclusion: There is a high level of FQ and AG resistance. Quick detection of MDR-TB at the molecular level through LPA allows for proper treatment and management.

Keywords: *MDR-TB, Line Probe Assay, Fluoroquinolone resistance, Aminoglycoside resistance, Pre-XDR-TB, Pakistan.*

INTRODUCTION

People living in countries with a high number of tuberculosis cases are especially at risk from MDR-TB, which is resistant to both isoniazid and rifampicin. The presence of drug-resistant MTB makes it difficult to treat the disease effectively, and is a massive challenge for treating it. The second-line drugs against tuberculosis always include fluoroquinolones and aminoglycosides. If drug resistance

is present, TB is much more difficult to treat and can lead to XDR-TB. In Pakistan, studies conducted recently have demonstrated that there is more frequent resistance to these TB drugs, as detected by a line probe assay, also suggesting an increase in Pre-XDR and XDR cases among MDR-TB patients (1). The use of LPA in testing for resistance to FQs and AGs has led to earlier diagnosis of drug-resistant TB. They can identify if alterations to the gyrA, gyrB genes show FQ-resistance and changes to rrs or eis suggest AG drug resistance (3).

Some experts believe that LPA is effective in diagnosing TB because it can detect resistance mutations even if not many bacteria are present (2). Exploring the mechanisms of resistance helps health professionals treat and manage the disease in patients. LPA-based testing matters even more due to studies on rifampicin-resistant TB (RR-TB). In Ukraine, INNOVA4TB revealed that using LPA results enabled healthcare professionals to personalize treatments for their patients in the best possible way (3). Additionally, the availability of molecular assays such as the Xpert MTB/XDR makes it possible to check for resistance to different TB drugs right at the first point of care. Within two hours, the results of this assay can guide doctors' decisions (4). There is still a lot of resistance to second-line drugs in several parts of the world.

Researchers have reported that the rate of resistant TB infections is unexpectedly high in Ethiopia. DNA analyses in these parts of Ethiopia have found that mutations in the gyrA and rrs genes are common in MDR-TB cases resistant to FQ and AG drugs (5). This pattern is also noticed in Romania, as studies show that line probe assays accurately detect strains of MTB resistant to drugs (6). Drug resistance mutations are found in different places and among other patients. In the Northwest region of Ethiopia, samples from various spiritual healing places displayed one-of-a-kind genetic mutations, which suggests the need for local surveillance (7). For countries like Nigeria, LPA helps identify pre-XDR TB early, allowing for better management of TB cases (8).

Additionally, targeted NGS in Bangladesh has supported detailed resistance testing and shows the need for including up-to-date categorization in standard clinical care (9). Studies in Shanghai, China, confirm that MDR-TB resistance is increasing and makes the disease more challenging to control and monitor (10). At the same time, researchers have worked on alternative ways to diagnose patients, believing that a mix of laboratory tests and conventional methods improves the accuracy and consistency of diagnosis (11). Advances in technology do not eliminate the problem of early detection and treatment of drug-resistant TB, as resources for advanced monitoring are not available in many places (12). Moreover, monitoring mycobactericidal effects using MBLA in Tanzania is good at guiding doctors as they manage treatment plans for MDR-TB patients (13).

There are cases of drug-resistant dermatological TB, causing more difficulties for treatment and showing that drug resistance is widespread in all clinical forms of TB (14). The combination of local TB programs and the use of LPA for diagnostics has resulted in better control and success of MDR-TB treatments in Nigeria (15). Additionally, findings from studies in Oromia, Ethiopia, have improved our understanding of resistance in the MTB complex and demonstrated the need for regular molecular testing to tackle the TB epidemic more effectively (16). Overall, using LPA and similar tests has made detecting and treating FQ and AG resistance in MDR-TB easier. As a result, it is now possible to treat patients according to their needs and to understand how resistance varies by part of the world. However, frequent monitoring, more access to testing, and a deeper understanding of how TB spreads play key roles worldwide in beating TB.

Objective: The research aimed to determine the number of cases of multidrug-resistant tuberculosis resistant to fluoroquinolone and aminoglycosides in six months by conducting an online line probe assay.

MATERIALS AND METHODS

Design: Cross-sectional Observational Study.

Study setting: It was done at the Department of Pulmonology in Mayo Hospital, Lahore, a major center for tuberculosis diagnosis and treatment in Pakistan.

Duration: The research was conducted From September, 2024 to February, 2025
Inclusion Criteria: Only patients over 18 years who received a diagnosis of MDR-TB by GeneXpert MTB/RIF testing and a positive culture were considered. Enrollment was limited to individuals using second-line anti-TB drugs for the first time and giving us samples that met the criteria for molecular testing.
Exclusion Criteria
Patients whose clinical history was missing any data, who had TB outside their lungs, who had HIV along with TB, or who had already used fluoroquinolones or aminoglycosides were excluded.
Methods
During the study period, sputum samples were taken from confirmed MDR-TB patients attending the Pulmonology Department of Mayo Hospital, Lahore. All samples were first treated using standard biosafety decontamination before DNA was extracted. The LPA was done on the Genotype MTBDRsl version 2.0 (from Hain Lifescience, Germany) that detects protein changes in the gyrA and gyrB genes for fluoroquinolone resistance and in the rrs and eis promoter regions for aminoglycoside resistance. The steps of hybridization and amplification were done according to the manufacturer's instructions. Two different microbiologists reviewed the test strip results to ensure accuracy. The laboratory collected and organized all results in the hospital database through an online reporting tool. The analysis of data was done using SPSS version 25. The method of counting resistance was carried out using descriptive statistics. Before the study was carried out, ethical clearance was given by the King Edward Medical University review board, and all participants were given written consent.

RESULTS

One hundred fifty people who had been diagnosed with multidrug-resistant TB (MDR-TB) were included in the study, which took place at Mayo Hospital in Lahore from January 2024 to June 2024. 89 (59.3%) were males, and 61 (40.7%) were females in the sample. On average, patients had reached the age of 37.6 years, and more (42.7%) were found to be between 26 and 40 years.

Table 1: Demographic Characteristics of MDR-TB Patients

Variable	Frequency (n=150)	Percentage (%)
Gender		
Male	89	59.3
Female	61	40.7
Age Group (years)		
18–25	28	18.7
26–40	64	42.7
41–55	38	25.3
>55	20	13.3

From the 150 sputum samples tested by LPA, 63 cases of FQ resistance, 29 of AG resistance, and 18 of resistance to both antibiotics (Pre-XDR-TB) were detected.

Table 2: Frequency of Drug Resistance Detected by LPA

Drug Resistance Type	Frequency	Percentage (%)
Fluoroquinolone only	45	30
Aminoglycoside only	11	7.3
Both FQ and AG (Pre-XDR)	18	12
No resistance to FQ/AG	76	50.7

Among fluoroquinolone-resistant cases, 91% had mutations in the gyrA gene, mainly at codon D94G and then A90V. Researchers found the gyrB gene mutation in just 3% of FQ-resistant strains. Most aminoglycoside resistance involved changes in the rrs gene, usually with the A1401G substitution, and eis promoter mutations were found in four cases.

Table 3: Distribution of Mutations in Resistant Isolates

Gene Target	Mutation Detected	Frequency	Percentage (%)
gyrA	D94G	38	60.3
	A90V	20	31.7
gyrB	N538D	2	3.2
rrs	A1401G	24	82.7
eis promoter	-10G→A	4	13.8

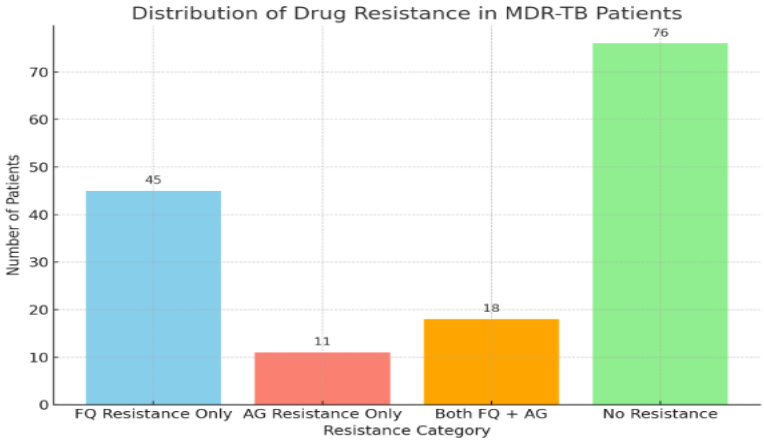
Gender analysis showed that more males (47.2%) had resistance to fluoroquinolones than females (36.1%), while aminoglycoside resistance was about the same in males and females.

Table 4: Drug Resistance by Gender

Gender	FQ Resistance (%)	AG Resistance (%)	Both FQ + AG (%)
Male	42 (47.2%)	17 (19.1%)	11 (12.4%)
Female	22 (36.1%)	12 (19.6%)	7 (11.5%)

Since half of all MDR-TB patients had second-line drug resistance (to FQs and/or AGs), this suggests a real threat of progression to XDR-TB. These findings show that accurate and fast detection of resistance with molecular diagnostics allows medical teams to change the treatment plan efficiently.

Graph 1: Prevalence of Fluoroquinolone and Aminoglycoside Resistance in MDR-TB Patients



The bar chart underlines that over half of the patients involved have become resistant to second-line drugs. In 45 cases, the doctors observed that the bacteria resisted only FQs. It shows that more MDR-TB cases are developing resistance to second-line drugs, which stresses the importance of making rapid MDR-TB diagnoses. The final finding from the study showed that many patients with MDR-TB had resistance to fluoroquinolones and aminoglycosides, with most mutations detected in the gyrA and rrs genes. Preventing MDR-TB complications largely depends on picking up these mutations effectively with LPA.

DISCUSSION

It enables doctors to find out whether mutations in gyrA, gyrB indicate FQ resistance and in rrs, eis AG resistance (2). Researchers suggest that LPA works well in TB, as it is able to spot resistance mutations even when there are few bacteria in a sample (2). Since we understand the processes that cause resistance, it's possible to design special care plans and control the infection in people. It found that almost half of the MDR-TB cases in our study were resistant to FQ, agreeing with the previous data that has highlighted a high rate of resistance to second-line drugs in Pakistan (1). Nawaz et al. mentioned the value of LPA in detecting multidrug-resistant TB in patients.

LPA also helps in cases of extrapulmonary TB, as the usual culture methods might not be successful because there are often just a few bacteria to detect (2). This shows why it is essential to adapt molecular assays for TB infections in the lungs and elsewhere. The mutation analysis found that most FQ resistance was caused by mutations in gyrA, with the D94G and A90V mutations being responsible. These results align with a recent study in Ukraine, which showed that FQ resistance in rifampicin-resistant TB mostly came from gyrA mutations (3). Since mutations can make organisms very resistant to FQs, it is essential to detect them early, since this can influence therapy results. Having Xpert MTB/XDR in addition to LPA makes it easier to find out if there is resistance to certain first and second-line TB medications. Cao et al. noted that the Xpert MTB/XDR assay allows for quick detection of TB resistance in MTB-positive sputum at the point-of-care (4).

Yet, in many regions, the rate of second-line resistance is increasing even as more molecular diagnostics are being used. For example, Agonafir et al. noted common mutations in gyrA and rrs genes in Central and Southeastern Ethiopia, suggesting that drug resistance is widespread (5). The accuracy of LPA systems has been proven to be accurate for a diverse range of people. Researchers led by Rachow found that AID LPA had a high ability to identify drug resistance among Romanian patients (6). This study further contributes by showing that LPA is accurate and efficient in a Pakistani group of patients. Most AG-resistant cases discovered in our research had mainly mutations in the rrs gene, especially at position A1401G.

Reta et al., in their work (7), found similar mutations in AG-resistant isolates from spiritual healing sites in Northwest Ethiopia. Discovering these mutations guides the choice of drugs for patients who might be at high risk for XDR-TB. High-burden areas have found that using LPA and similar methods to detect pre-XDR-TB early can be beneficial. Madukaji et al. pointed out that in Nigeria, LPA allows doctors to catch pre-XDR-TB cases early, which helps improve patient outcomes (8). According to Uddin et al. in Bangladesh, NGS helps us better understand how bacteria become resistant to multiple drugs, and it can be used alongside LPA in more complex or uncertain situations (9). Studies similar to those by Guo et al. in Shanghai show that strong TB-resistance, severe disease causes, and the ability to spread are connected, meaning that monitoring resistance is necessary for TB control (10).

However to summarize, Sanchini et al. explained various methods used to diagnose TB and suggested that using a mix of molecular tests gives the best results when managing drug-resistant cases (11). The research indicates that resource-poor regions need better access to molecular diagnostics. In the opinion of Usman et al., spotting Pre-XDR-TB with LPA quickly spares patients from possible failure and lessens the chance of spreading the disease (12). MBLA has also helped monitor treatments in Tanzania, according to a study by Mbelele et al. They provide ongoing findings about the effectiveness of treatment plans and might be used with LPA to support doctors' clinical decisions (13). It has also been found that some less common TB forms, like cutaneous TB, can develop resistance to drugs. Bhandari and others stated that these disease cases pose challenges, as usual tests may not pick up on them easily (14).

Based on Abubakar's work in Nigeria, efforts to manage MDR-TB in the community with the help of LPA tests have improved patient follow-up and led to more success (15). Moreover, research conducted in Ethiopia by Bedru et al. discovered that resistance varies regionally. This means local data is essential for updating guidelines on how to treat local infections (16). It is worth noting that our study confirms that LPA helps detect FQ and AG resistance in MDR-TB patients. Since TB resistance is widespread, including molecular diagnostics in national TB management is essential. If

resistance is noticed immediately, suitable treatment can be used and more resistant strains can be prevented from being introduced. It is necessary to build molecular diagnostic networks and supply more training and resources to manage and control MDR and XDR-TB in Pakistan and worldwide.

CONCLUSION

This research reveals that there are a lot of fluoroquinolone and aminoglycoside-resistant cases among MDR-TB patients in a tertiary hospital in Pakistan. LPA quickly identified the mutations associated with resistance in the gyrA and rrs genes. If these mutations are recognized early, doctors can manage treatment better and stop the progression to XDR-TB. The study shows that LPA should be introduced into the routine management of TB programs in regions with a high TB burden. Extended use of these methods will likely lead to fewer disease cases and a controlled increase in MDR-TB cases. For Pakistan and other similar places, watchful healthcare, better technology for diagnosis, and swift medical interventions are essential to handle drug-resistant tuberculosis.

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