Pak. J. Chest Med. 2024;30(03)

Original Article

journal homepage: https:/www.pjcm.net/

Pakistan Journal of Chest Medicine

Official journal of Pakistan Chest Society

Evaluation of Drug Resistance Patterns in Mycobacterium tuberculosis Isolates from Tuberculosis Patients

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Article History:

Received:	Jan 17, 2024
Revised:	Apr 15, 2024
Accepted:	July 01, 2024
Available Online:	Sep 02, 2024

Author Contributions:
GZA conceived idea, IUW drafted the study, MI collected data, ST did statistical analysis and interpretation of data, WZA GZA critical reviewed manuscript. All approved final version to be published.

Declaration of conflicting interests:
The authors declare that there is no conflict of interest.

How to cite this article:
Afridi GZ, Wazir IU, Tariq S, Ilyas M, Afridi WZ. Evaluation of Drug Resistance Patterns in Mycobacterium tuberculosis Isolates from Tuberculosis Patients. Pak J Chest Med. 2024;30(03):333-339

ABSTRACT

Background: Tuberculosis (TB) remains a major global health challenge, particularly in high-burden countries like Pakistan. The emergence of drug-resistant Mycobacterium tuberculosis (MTB) strains has severely compromised treatment success. Early detection of resistance to first-line anti-TB drugs is crucial for effective management.

Objective: To determine drug susceptibility patterns of Mycobacterium tuberculosis isolates from Tuberculosis patients.

Methodology: This study included 280 patients with confirmed pulmonary TB from January to December 2023. We processed sputum samples using the NALC-NaOH method and cultured them on Lowenstein-Jensen medium. We tested for drug susceptibility to isoniazid, rifampicin, ethambutol, and streptomycin using the proportion method. Data was analyzed with SPSS version 26.0, setting the significance level at $p < 0.05$.

Results: Out of 280 isolates, 96 (34.3%) showed resistance to at least one first-line anti-TB drug. Isoniazid resistance was most common (16.4%), followed by streptomycin (13.6%), ethambutol (11.8%), and rifampicin (4.3%). Multidrug-resistant TB (MDR-TB) was detected in 6.1% of cases, with significantly higher prevalence among previously treated patients ($p = 0.001$).

Conclusion: The present study concluded that anti-TB drug resistance is very important in TB patients, especially in those who have been treated previously. In the present study, Isoniazid resistance was particularly common, showing the importance of regular drug susceptibility testing. Early detection and customized treatment plans are vital to prevent the spread of resistant TB strains. Improving diagnostic capacity is crucial for effective TB control.

Keywords: Tuberculosis; Mycobacterium Tuberculosis; DST; First Line Anti-TB Drugs

Introduction

Tuberculosis (TB) is an infectious disease caused by a bacterium known as Mycobacterium tuberculosis (MTB). Primarily TB affects the lungs, but it can also involve other parts of the body. TB spreads through the air when a person with active pulmonary TB coughs, sneezes, or speaks, releasing infectious droplets that can be inhaled by others nearby. Crowded living conditions, poor ventilation, and prolonged close contact increase the risk of transmission. Despite being preventable and treatable, TB remains a major public health concern, especially in densely populated and resource-limited settings. In 2022, the World Health Organization (WHO) estimated that 10.6 million people were diagnosed with TB, and 1.3 million died from it. This makes TB the second leading cause of death from an infectious agent, just after COVID-19.[1] Notably, Pakistan is one of the top five countries with a high burden of TB, significantly adding to the global TB cases.[2] Although TB can be prevented and treated, the rise of drug-resistant forms, especially multidrug-resistant TB (MDR-TB), poses a major threat to efforts to control the disease globally.

TB is treated through specialized treatment program known as directly observed treatment short courses (DOTs) under the national TB control program. Different first line drugs known as First-line anti-TB drugs are used to treat TB under TB control program. First line drugs are isoniazid (INH or H), rifampicin (RMP or R), ethambutol (E), and pyrazinamide (PZA or Z). In many parts of the world TB is somewhat controlled through this program but unfortunately resistance occurred against different drugs which cause severe issues and may patients fail to achieve successful treatment outcome. Different level of resistance to these drugs occurred which include monoresistance, poly-drug resistance and multidrug resistance. Among drug resistance Multidrug Resistance is most prevalent and difficult to treat and has reduced treatment effectiveness and increased transmission risks.[3] Worldwide, an estimated 3.4% of new TB cases and 18% of previously treated cases are MDR-TB, with about 410,000 new MDR/RR-TB cases arising in 2022.[1,4] Additionally, isoniazid-resistant TB (Hr-TB) makes up over 11% of the global TB burden. Managing Hr-TB is challenging due to limited diagnostic screening in many resource-poor areas.[5]

Resistance can be categorized as either primary (transmitted) or acquired (developed during treatment). Several factors lead to the development of resistance. These include poor adherence, inappropriate treatment plans, weak healthcare systems, and limited access to drug susceptibility testing (DST). Although molecular diagnostic tools like GeneXpert MTB/RIF have improved the early detection of rifampicin resistance, they do not identify resistance to other first-line drugs, such as isoniazid, ethambutol, and streptomycin.[6] Consequently, patients who are resistant to these drugs may receive less effective treatment, resulting in treatment failure, relapse, and a greater spread of resistance.[7] This situation underscores the need for regular phenotypic DST, particularly in cases that have been treated before, where the likelihood of resistance is much higher.

A 2023 global review examined more than 148 studies. It found an overall MDR-TB prevalence of 11.6%. This rate varies by region, previous treatment history, and the lab methods used.[8] Furthermore, research from South Asia indicates that isoniazid monoresistance is increasing, even among new TB cases. This issue is often overlooked because of the dependence on rifampicin-only detection tools.[9] These results point to changes in resistance patterns. Regional evaluations are needed to adjust diagnostic and treatment strategies effectively.

Despite Pakistan's classification as one of the countries with the highest burden of TB, there is a lack of published research on the full range of resistance to first-line anti-TB drugs, particularly among different treatment history groups. This study aimed to examine drug resistance patterns to isoniazid, rifampicin, ethambutol, and streptomycin in M. tuberculosis samples collected from newly diagnosed and previously treated TB patients. By offering updated and region-specific resistance profiles, this study seeks to guide treatment policies, improve clinical decision-making, and strengthen public health efforts in TB control.

Objective

To determine drug susceptibility patterns of Mycobacterium tuberculosis isolates from Tuberculosis patients.

Methodology

This was a laboratory study conducted over six months, from January to December 2023. The study took place at the TB Centre, Hayatabad Medical Complex, Peshawar. Hayatabad Medical Complex also has a top facility for diagnosing and managing tuberculosis. The laboratory has a biosafety level 3 (BSL-3) infrastructure and is certified for TB culture and drug susceptibility testing.

In the present study, a total of 280 sputum samples were collected from pulmonary tuberculosis (PTB) patients with confirmed TB. Patients were enrolled consecutively from outpatient and inpatient departments. The inclusion criteria were age 18 years or older, a confirmed diagnosis of pulmonary TB through sputum smear microscopy and/or GeneXpert MTB/RIF, and no prior use of second-line anti-TB drugs. Patients were divided into new cases, meaning they had never received TB treatment or had been treated for less than one month, and previously treated cases, which included patients with a history of TB treatment for more than one month.

Early morning sputum samples were collected from each

participant in sterile, wide-mouthed containers. The samples were taken to the microbiology lab under cold-chain conditions and processed the same day using the standard N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) decontamination method. After decontamination and concentration, sediment from each sample was placed into Lowenstein-Jensen (LJ) medium and incubated at 37°C for up to eight weeks. We regularly checked for colony growth.

Growth on LJ medium confirmed the presence of Mycobacterium tuberculosis complex (MTBC) based on colony appearance, slow growth rate, and biochemical tests. These tests included niacin accumulation, nitrate reduction, and catalase activity at 68°C. Isolates that displayed typical MTBC traits were chosen for drug susceptibility testing. We performed DST using the proportion method on LJ medium, following WHO guidelines. Each isolate was tested against the four first-line anti-TB drugs: isoniazid (0.2 µg/mL), rifampicin (40 µg/mL), ethambutol (2 µg/mL), and streptomycin (4 µg/mL). A strain was labeled resistant if 1% or more of the bacillary population grew on media containing the drug, compared to the drug-free control.

We used the reference strain Mycobacterium tuberculosis H37Rv (ATCC 27294) as a quality control for both culture and DST procedures. All lab work followed biosafety guidelines and internal quality assurance protocols. External quality assessment took place periodically by the national TB reference laboratory.

Data on patient demographics, clinical history, and DST results were recorded on pre-structured forms and entered a secure database. We performed statistical analysis using SPSS version 26.0. We calculated frequencies and percentages for categorical variables and means with standard deviations for continuous variables. The chi-square test was used to compare resistance rates between new and previously treated cases, with $p < 0.05$ regarded as statistically significant.

Results

In the present study, a total of 280 culture-positive tuberculosis (TB) patients were included. Among the study case, majority were male (162, 57.9%) with a male-to-female ratio of 1.4:1 (Figure 1).

Results showed that, the age of participants ranged from 18 to 74 years with a mean age of 36.8 ± 11.7 years. Most of the participants (44.3%) belonged form age group of 25–44 years. Regarding educational background, primary-level education was the most common (40.4%), followed by secondary (36.1%), and tertiary (23.5%). Employment status varied; 98 (35%) were unemployed, 87 (31%) were self-employed, and 95 (34%) held formal employment.

Of the total sample, 221 (78.9%) were newly diagnosed TB cases, and 59 (21.1%) were previously treated (Figure 2).

All MTB isolates were tested for Drug susceptibility testing (DST). DST was performed for isoniazid (H), rifampicin (R), ethambutol (E), and streptomycin (S) and results showed that among the newly diagnosed TB patients, 171 (77.4%) were fully susceptible to all drugs, whereas 50 (22.6%) had resistance to at least one drug. In contrast, only 13 (22.0%) of the previously treated patients were fully susceptible, and 46 (78.0%) exhibited resistance to one or more drugs.

DST showed that 16.4% of the isolates showed resistance to Isoniazid, 11.8% to Ethambutol, 13.6% to Streptomycin and 4.3% to Rifampicin. Isoniazid monoresistance was more pronounced among previously treated individuals (28.8%) compared to new cases (12.2%), $p = 0.006$. Streptomycin resistance was also significantly higher in previously treated cases ($p = 0.013$).

Among the study cases, Multidrug-resistant TB (MDR-TB) was observed in 17 cases (6.1%). The prevalence of MDR-TB was markedly higher among previously treated cases (10/59; 16.9%) than among newly diagnosed patients (7/221; 3.2%), and this difference was statistically significant ($p = 0.001$).

The most frequent dual-drug resistance patterns included isoniazid + ethambutol (H + E) in 6.8% and isoniazid + streptomycin (H + S) in 5.4% of the total cohort. Triple-drug resistance (H + R + E) was found in 2 patients (0.7%), while quadruple resistance (H + R + E + S) was not observed (Table 2).

Discussion

This study examines drug resistance patterns in Mycobacterium tuberculosis samples from 280 culture-confirmed TB patients. The results show a significant level of drug resistance, especially in those who have been treated before. By closely looking at the rates of monoresistance and multidrug resistance (MDR) and comparing the susceptibility profiles of new and retreatment cases, our findings shed light on the current challenges in controlling tuberculosis.

In the present study, we found that 34.3% of TB isolates showed resistance to at least one first-line drug. This results was in line with the findings of other studies, like a meta-analysis conducted by Bastos et al., 2017 pointed out that isolates from eastern Iran reported an overall resistance rate of 31.0% among first-line drugs, which closely matches our findings.[10] Another study in a tertiary-care center in Pakistan found 29.6% resistance to any first-line agent (Javaid et al 2020).[11] These similarities underline a continued and widespread issue across various regions, emphasizing the need for improved surveillance and universal DST for all TB cases.

Results of the present study showed that INH monoresistance was observed in 16.4% of the isolated MTB. This rate was near to the global pooled INH

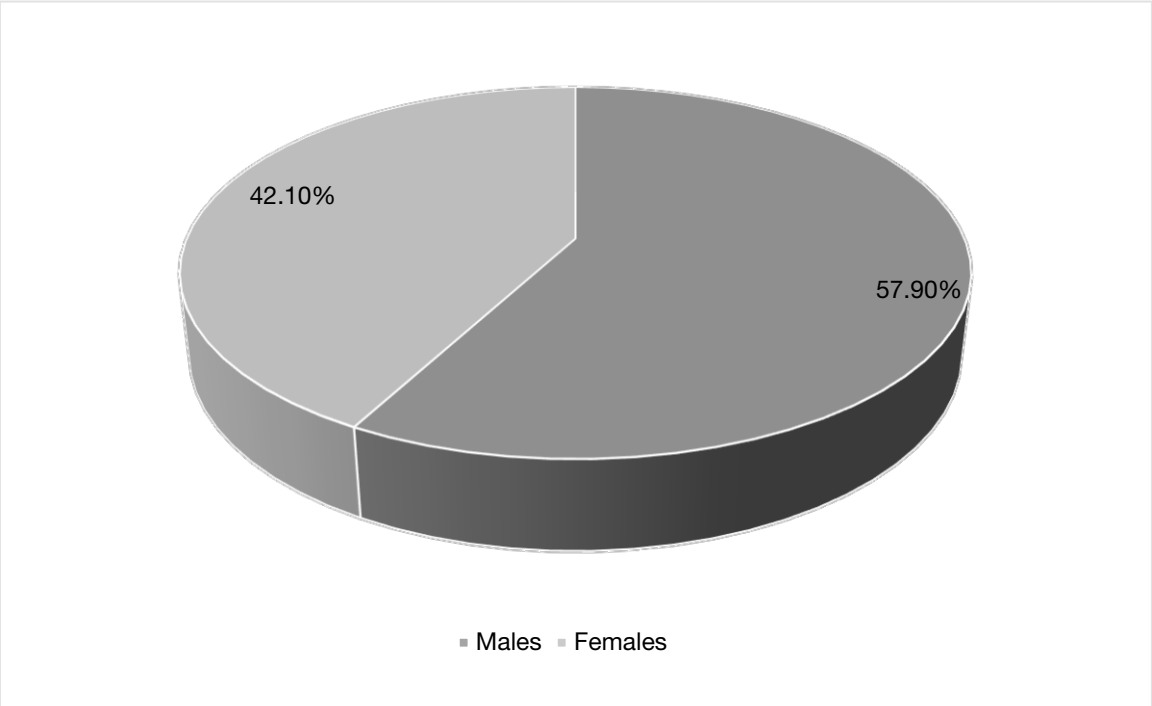

Figure 1. Gender Base distribution of study cases

resistance rate, which was reported as 15.7% in a study by Varaharam et al., 2014.[12] A multi-country study conducted by Shaivekar et al., 2020 also found the INH monoresistance of 11.6%, which is slightly lower than our rate, especially in previously treated cases.[13] Our higher rate may be due to regional treatment dynamics or drug use practices. Since INH monoresistance is linked to poor treatment outcomes, with success rates reported as low as 62-78% (Nagar et al., 2022),[14] our findings highlight the need for early molecular detection, such as katG and inhA mutation assays. We may also need to consider using levofloxacin-based regimens for improved results.

Overall, the prevalence of MDR-TB in this group was 6.1%. We saw significant differences between new cases, which were at 3.2%, and previously treated cases, which were at 16.9%. Worldwide, the MDR prevalence is about

Table 1. Resistance Patterns in M. tuberculosis Isolates (n = 280)

Resistance Type	Newly Diagnosed (n=221)	Previously Treated (n=59)	Total (n=280)
Sensitive to All Drugs	171 (77.4%)	13 (22.0%)	184 (65.7%)
Any Resistance	50 (22.6%)	46 (78.0%)	96 (34.3%)
Monoresistance			
Isoniazid	27 (12.2%)	19 (28.8%)	46 (16.4%)
Rifampicin	4 (1.8%)	8 (13.6%)	12 (4.3%)
Ethambutol	20 (9.0%)	13 (22.0%)	33 (11.8%)
Streptomycin	24 (10.9%)	14 (23.7%)	38 (13.6%)
MDR-TB (H + R)	7 (3.2%)	10 (16.9%)	17 (6.1%)

Table 2. Patterns of Combined Drug Resistance

Drug Resistance Pattern	Frequency (n=280)	Percentage (%)
H + E	19	6.8
H + S	15	5.4
R + S	5	1.8
R + E	4	1.4
H + R + E	2	0.7
H + R + E + S	0	0.0

3.4% in new cases and 18% in retreatment cases (Munir et al., 2019).[15] In sub-Saharan Africa, the pooled MDR rate among new TB patients was estimated at 2.0% (Musa et al., 2017).[16] Our results match global averages well. They especially show that treatment history is a strong risk factor for MDR, which aligns with WHO data and several meta-analyses. This highlights the urgent need to focus on DST and monitor adherence, particularly in retreatment cases.

We found high resistance rates to streptomycin (23.7%) and ethambutol (22.0%) among patients who had been treated before. A study from Eastern Iran showed similar trends, with resistance rates of 20% for streptomycin and 19% for ethambutol. In Nepal, resistance among patients needing retreatment reached around 18% to 22% (Tharu et al., 2014).[17] These results indicate that relying only on H and R susceptibility for these cases may not lead to the best treatment plans. We suggest expanding DST panels to include S and E for better therapeutic planning.

Additionally, the much higher resistance rates in previously treated patients highlight the ongoing effects of incomplete treatment, self-medication, and poor

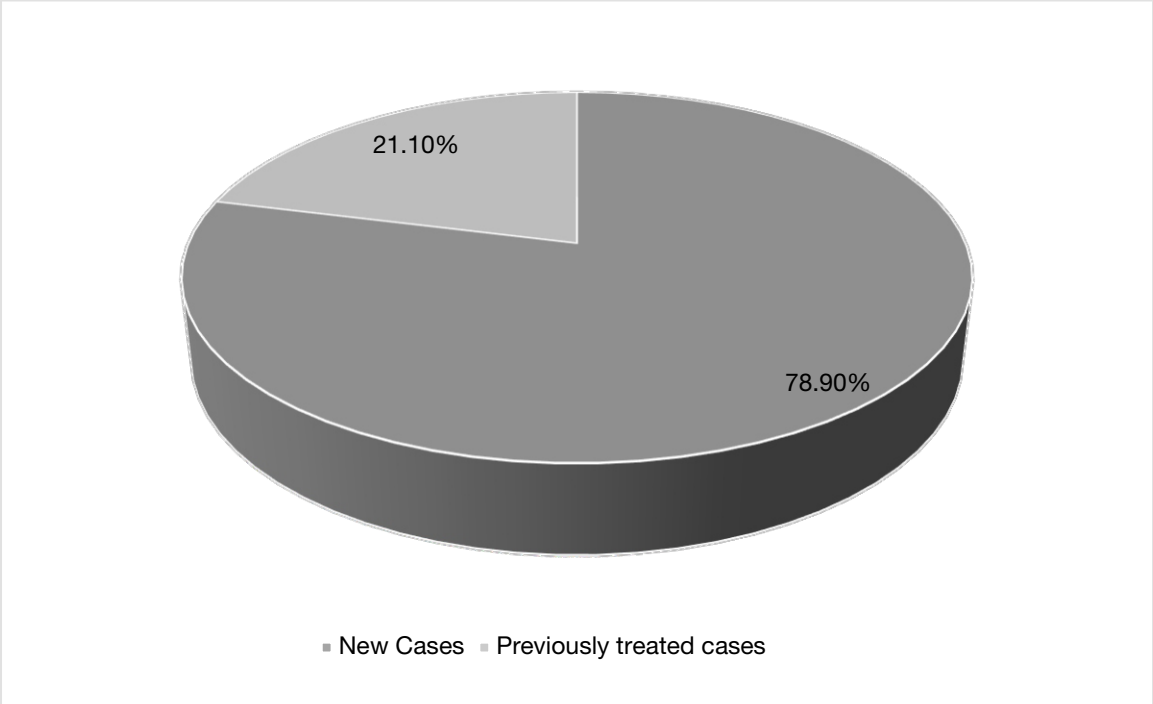

Figure 2. Distribution of cases on bases of treatment history

adherence. This suggests that many retreatment cases may have gained resistance during earlier treatments due to inconsistent drug supply, lack of follow-up, or unnoticed treatment failure. This trend, which matches findings from several regional studies, shows the need for proper case tracking, clear retreatment guidelines, and universal drug susceptibility testing in national TB control strategies. The observed rates of isoniazid and streptomycin resistance also reflect the continued circulation of strains resistant to older first-line drugs, especially in settings where empirical therapy is common without prior DST. These results reinforce the need for wider access to rapid molecular diagnostic tools, such as GeneXpert MTB/RIF and line probe assays, not only for rifampicin resistance detection but also for early identification of isoniazid and other first-line drug resistance. Expanding such diagnostic capacity will support more targeted treatment regimens and reduce the risk of generating further resistance.

Lastly, our findings suggest that the current standard first-line regimens may not be suitable everywhere, especially in areas with high rates of isoniazid or ethambutol resistance. We need personalized treatment strategies that take local DST data into account and are backed by practical research to improve outcomes. We must strengthen laboratory infrastructure, invest in training for healthcare providers, and make DST a normal part of TB care, particularly for retreatment and high-risk groups. This is essential to combat the ongoing threat of drug-resistant TB and achieve global elimination targets.

Conclusion

This study shows a high rate of drug-resistant Mycobacterium tuberculosis in both new and previously treated TB patients. The retreatment group had much higher resistance rates. Isoniazid was the most commonly resistant drug, followed by streptomycin, ethambutol, and rifampicin. The finding of multidrug-resistant TB in 6.1% of cases, especially among those previously treated, highlights the urgent need for regular drug susceptibility testing as part of initial TB evaluation. Customized treatment plans based on DST results are crucial to improving patient outcomes, preventing treatment failure, and reducing the spread of resistant TB strains in the community.

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