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PREVALENCE OF MULTIDRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* AMONG PATIENTS VISITING TB HOSPITAL KHAIRPUR

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Abstract

Multidrug-resistant tuberculosis (MDR-TB) remains a major public health concern, particularly in developing countries such as Pakistan, where delayed diagnosis and inadequate treatment contribute to ongoing transmission. This study aimed to determine the prevalence of Mycobacterium tuberculosis (MTB) and MDR-TB among suspected tuberculosis patients using the GeneXpert MTB/RIF assay. A total of 240 clinical samples were analyzed, of which 53 (22.08%) were confirmed positive for MTB. Among these, 10 cases (18.87%) were identified as rifampicin-resistant and were classified as MDR-TB due to the well-established association between rifampicin and isoniazid resistance. Age-wise analysis revealed that the highest proportion of MTB-positive cases was observed in the 51-65 years age group (34.78%), followed by 36-50 years (26.92%), 21-35 years (19.35%), and 6-20 years (16.22%), while no cases were detected in individuals above 65 years. Statistical analysis using Fisher's Exact Test indicated no significant association between age group and MTB positivity (p =0.091) or rifampicin resistance (p= 0.87), Gender-based analysis showed a higher prevalence of MTB among male (60.4%) compared to females (39.6%). Similarly, rifampicin resistance was more frequent in males (70%) than females (30%). Taluka-wise distribution demonstrated that Khairpur had the highest percentage of MTB-positive cases (44%), while Naro reported the lowest (10%). Rifampicin resistance was highest in Kotdiji (28.57) and lowest in Kingri (11.11%). In conclusion, the study highlights a considerable burden of MTB and MDR-TB in the region, emphasizing the need for early diagnosis, targeted screening, and strengthened tuberculosis control strategies,

1. INTRODUCTION

Tuberculosis (TB) continues to be a prominent infectious threat globally. Recently, the WHO documented 10.88 million fresh TB cases worldwide in 2023 (Goletti et al., 2025). More than two-thirds of cases were attributed to eight countries, with Pakistan responsible for 6.3% of the worldwide TB burden (Karim et al., 2023). Drug-resistant tuberculosis, particularly multidrug-resistant tuberculosis (MDR-TB), characterized by resistance to at least isoniazid and rifampicin, presents a significant obstacle for control initiatives. In 2022-2023, the worldwide incidence of MDR/RR-TB was estimated to be 410,000 cases annually (Song et al., 2024), and around 3.3% of new TB cases globally are

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MDR/RR-TB (Xi et al., 2022). In Pakistan, the occurrence of TB is still elevated, and there is an increasing trend in drug resistance

The Khairpur District in Sindh, a key rural area, is regarded as a high-burden TB region with socio-economic obstacles to healthcare access and postponed diagnosis. Data on MDR-TB in Khairpur is limited. Since rifampicin resistance serves as a dependable indicator of MDR-TB, molecular identification of rpoB gene mutations (notably in the 81-bp rifampicin-resistance-determining region, RRDR) can quickly recognize resistant strains. The rpoB gene encodes the β-subunit of RNA polymerase, which is the target of rifampicin; mutations in rpoB (e.g., Ser531Leu, Asp516Val, His526Asp/Tyr) are recognized to provide strong resistance (Portaels, 2024).

This study was aimed to determine the prevalence of TB and MDR-TB among patients at TB Hospital Khairpur. The current study integrated conventional diagnostics (smear microscopy) with molecular techniques (GeneXpert) to derive epidemiological and genetic insights into TB in this setting. Such data are critical for guiding treatment strategies, strengthening local TB control, and contributing to the global effort to eliminate TB.

2. Material and Methods

The study was conducted at the TB Hospital, Khairpur Medical College, Khairpur Mirs, after obtaining ethical approval from the Health Department of the Government of Sindh. Written informed consent was secured from all participants or their guardian prior to sample collection. A total of 240 suspected tuberculosis (TB) patients were included, based on a sample size calculated using a 19% prevalence of multidrug-resistant TB at 95% confidence level. Patients were selected according to defined inclusion and exclusion criteria (Mughal et al.,2025).

All enrolled patients underwent a detailed clinical examination to assess symptoms such as persistent cough, fever, weight loss, and hemoptysis, followed by chest X-ray evaluation interpreted by a qualified radiologist. Demographic and clinical information was systematically recorded using a standardized proforma in accordance with National TB Control Program (NTB) guidelines.

Sputum samples were collected following standard procedures, including spot and early morning specimens. Acid fast Bacilli (AFB) microscopy was performed (for follow up cases) using Auramine O fluorescence staining for initial diagnosis. Samples (all new and positive AFB samples) were further processed for GeneXpert (IUATLD, 2013: WHO, 2014). All laboratory procedures were carried out in Biosafety Level 2 (BSL-2) facilities with strict adherence to safety protocols, including the use of personal protective equipment and biological safety cabinets.

3. Results

3.1 Physical Examination

In this study, a total of 240 patients visiting TB hospital during research period were included. The majority of patients presented with moderate to severe cough (n=237), followed by low grade or intermittent fever, nausea, weight loss, vomiting, hemoptysis, co-morbidity, and hypertension (Figure.1). Gender wise appearance of sign and symptoms in percentage (%) in mentioned in Figure 2.

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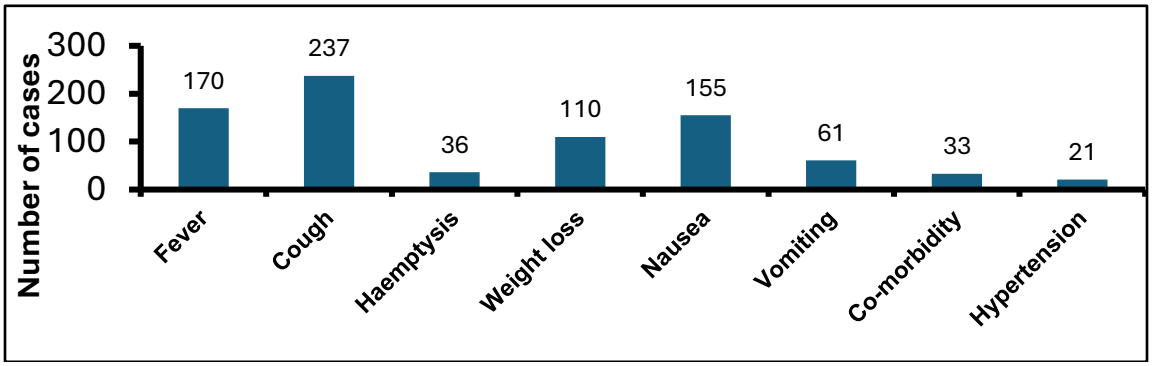


Figure 1. Frequency of particular sign and symptom in patients

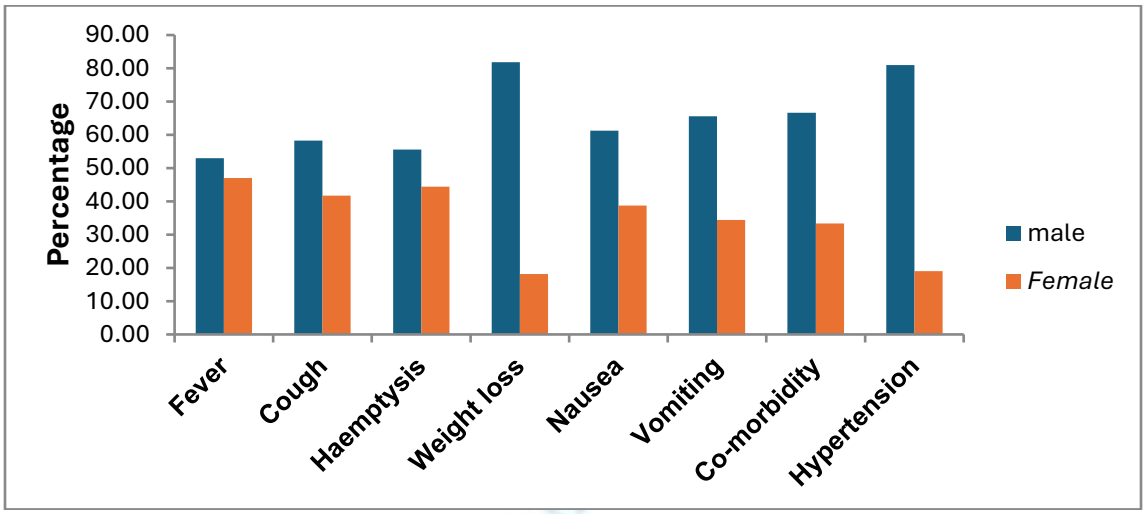


Figure 2. Gender wise appearance of sign and symptoms

3.2 Chest X-ray

Following physical examination, patients were referred for X-ray investigations. X-ray illumination either showed normal view or typical findings for M. *tuberculosis* including cavitations, consolidations, and infiltration. Figure 3 A Shows bilateral consolidation in lower lobes major on right side, obliteration of right costeophrenic angle, enlarged lymph nodes: Figure 3 B shows bilateral consolidation at bilateral upper lobes. Figure 3 C shows right middle lobe consolidation and large cavity at left upper lobe. Figure 3 D shows infiltration at right middle lobe and cavity with adjacent fibrosis at left upper lobe.

Figure 3 E shows cavity and fibrosis at left upper lobe; Figure.3 F shows bilateral loss of lung volumes with large right middle lobe consolidation and left fibrosis and suspected pleural effusion. Figure 3 G shows cavity and fibrosis at left upper lobe and figure 3 H shows bilateral loss of lung volumes with large right middle lobe consolidation and left fibrosis and suspected pleural effusion.

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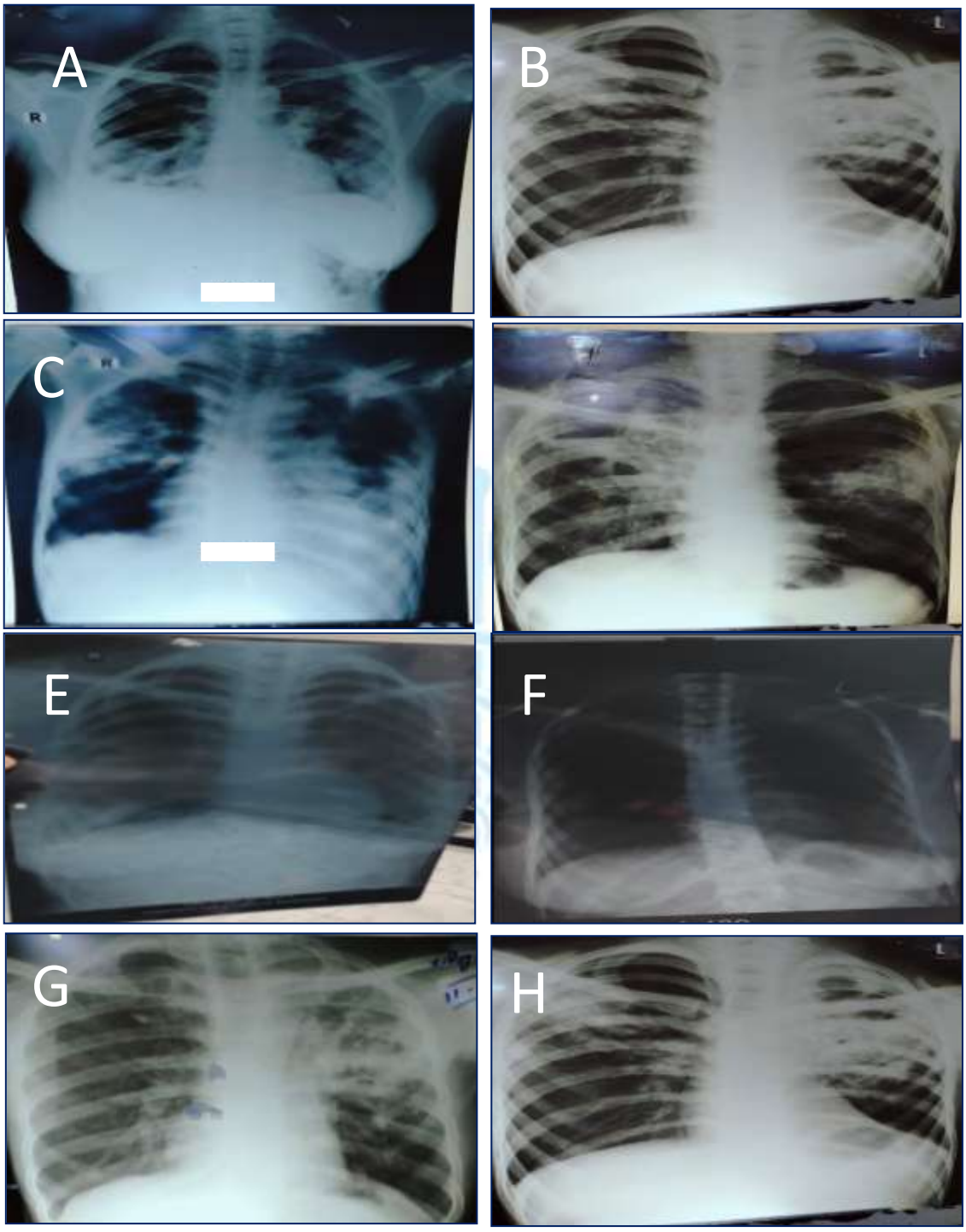


Figure 3.Chest X-ray findings in patients with suspected tuberculosis

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3.3 AFB Microscopy

Since the prevalence includes the old as well as new cases, in this study sputum samples of old cases/follow up cases (n=74) were processed for sputum AFB microscopy. The International Union against Tuberculosis and Lung Disease (IUATLD) and World Health Organization (WHO) criteria for national TB programs were followed in counting the amount of AFB. The samples showed (Figure 4) varied range of AFB load per field from not detected to >60 AFB in one field (grading +3). Number and percentage of male and female cases in each grading is mentioned in Table 1.

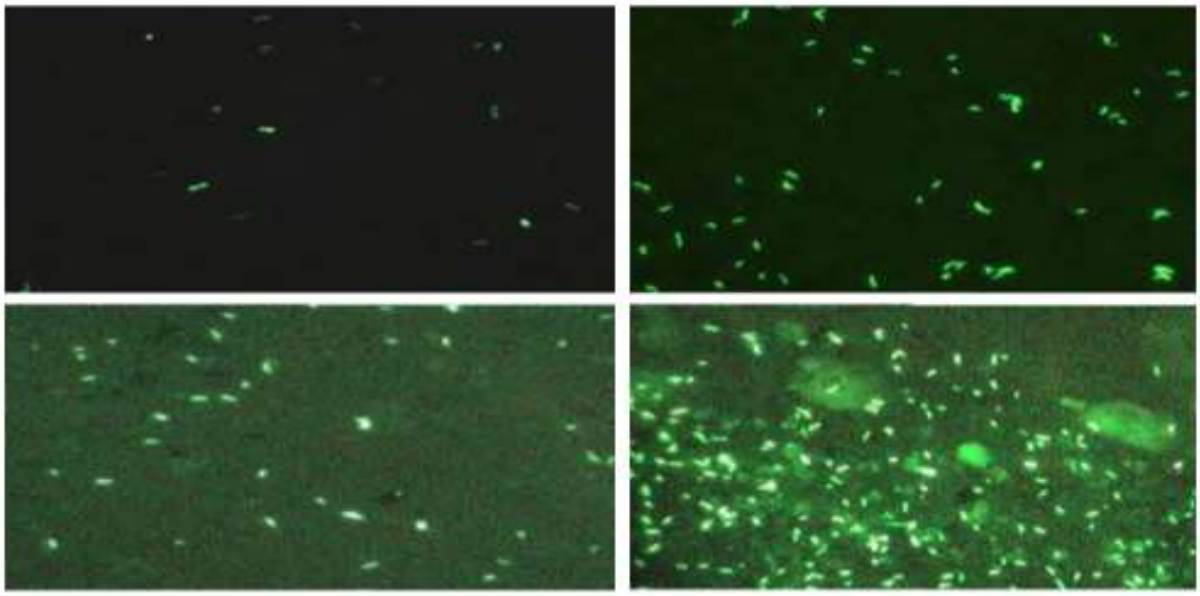


Figure 4.Fluorescence microscopy of *M. tuberculosis* using Auramine O staining

Table 1. Grading criteria for AFB sputum smear and number of cases

Number of AFB (400X)	Grading	Number of Cases (Male, %)	Number of Cases (Female, %)
No AFB in one length	Negative	24 (32.43%)	14 (18.92%)
3–24 AFB in one length	Scanty	11 (14.86%)	7 (9.46%)
1–6 AFB in one field	1+	7 (9.46%)	5 (6.76%)
7–60 AFB in one field	2+	1 (1.35%)	2 (2.70%)
>60 AFB in one field	3+	2 (2.70%)	1 (1.35%)
Total	–	45 (60.8%)	29 39.2%)

3.4 GeneXpert Analysis and Prevalence of *M. tuberculosis* and MDR-TB

Out of a total of 240 samples tested using the Gene-Xpert assay, 53 samples (22.08%) were found to be positive for *Mycobacterium tuberculosis* (MTB). Among these 53 MTB-positive cases, 10 samples (18.87%) were identified as Rifampicin-resistant (Table 2). According to existing literature, Rifampicin resistance is strongly associated with resistance to Isoniazid (INH), another key first-line anti-tuberculosis drug. Therefore, these 10 Rifampicin-resistant cases were classified as Multi-Drug Resistant Tuberculosis (MDR-TB) (Table 2).

An age-wise analysis of the samples showed that the highest proportion of MTB-positive cases occurred in the 51–65 year age group (34.78%), followed by the 36–50 year age group (26.92%). The 21–35 year age group accounted for 19.35% of cases, while 16.22% were observed in the 6–16

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year age group. Notably, no MTB-positive cases were detected among individuals aged above 65 years. Fisher Exact test performed showed that there was no significant association between age group and MTB positivity (p=0.091) and age group and rifampicin resistance (p=0.87) respectively.

Table 2. Age wise MTB positive cases and MDR cases

S. No	Age group (Years)	Total Samples	MTB +ve cases and %	RIF Resistant/MDR cases and %
1	6-20 years	37	6 (16.22)	1 (16.66)
2	21-35 years	93	18 (19.35)	3 (16.66)
3	36-50 years	78	21 (26.92)	5 (23.81)
4	51-65 years	23	8 (34.78)	1 (12.5)
5	>65 years	9	0 (0)	0 (0)
	Total	240	53 (22.08)	10 (18.87)

Gender-wise analysis of the 53 MTB-positive cases showed that 32 cases were male, while 21 cases were female. Among the 10 Rifampicin-resistant cases, 7 cases (70%) were male, and 3 cases (30%) were female. The age and gender-wise distribution of MTB-positive and Rifampicin-resistant cases is illustrated in Figure 5.

A taluka-wise analysis of the data revealed that Khairpur Taluka reported the highest percentage of MTB-positive samples at 44%, while the lowest percentage (10%) was recorded in Naro Taluka. In terms of Rifampicin resistance, the highest percentage (28.57%) was observed in Kotdigi Taluka, whereas Kingri Taluka had the lowest percentage at 11.11%. The detailed taluka-wise distribution of MTB-positive cases and Rifampicin-resistant cases is presented in Table 3.

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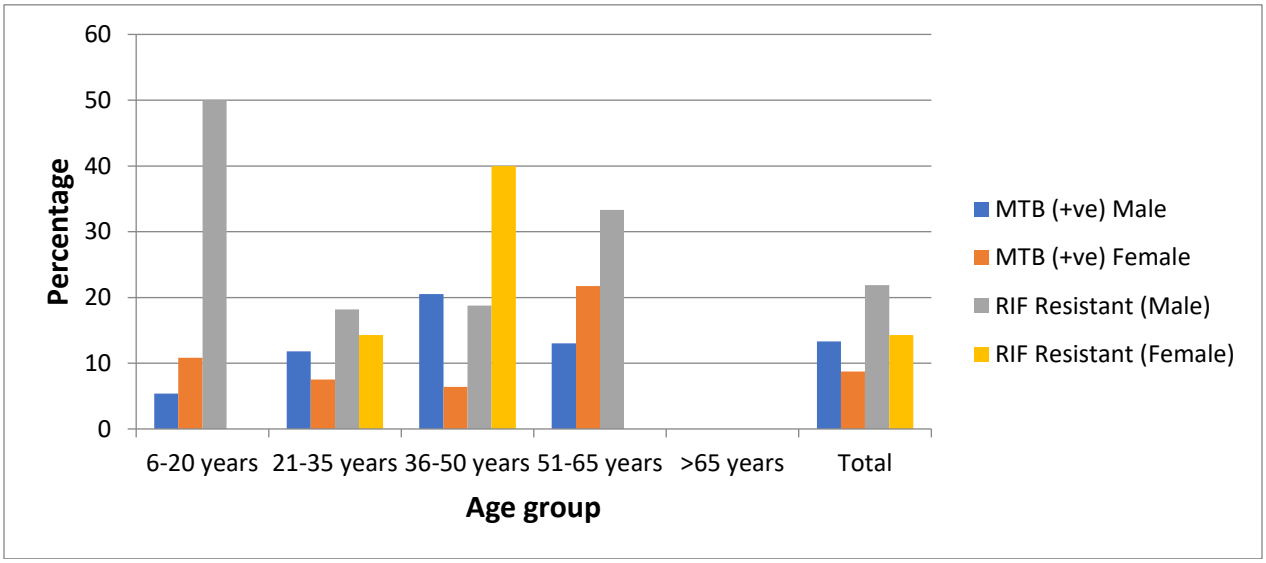


Figure 5. Age and Gender wise analysis of MTB and Rif resistant cases

Table 3. Taluka-wise Distribution of MTB and Rifampicin-resistant Cases

S.No:	Name of Taluka	Total samples	MTB (+ve)	(%)	Rif Resistant cases	(%)
1	Khairpur	25	11	44	3	27.27
2	Kotdigi	35	7	20	2	28.57
3	Naro	31	3	10	0	0
4	Gambat	40	8	20	1	12.5
5	Kingri	30	9	30	1	11.11
6	Sobhodero	25	4	16	1	25
7	ThariMirwah	30	6	20	1	16.66
8	Faizganj	24	5	21	1	20
Total		240	53	22.08	10	18.87

4. Discussion

The current research offers significant understanding regarding the occurrence of M. tuberculosis and multidrug-resistant tuberculosis in patients visiting a TB Hospital in Khairpur Sindh. The total MTB positivity rate of 22.08% noted in this research indicates a significant impact of tuberculosis in the area. This result aligns with previous reports from high-burden areas in Pakistan, where socio-economic difficulties, overcrowding, and postponed healthcare-seeking behavior lead to ongoing transmission (Ali et al., 2023). The noted prevalence highlights that TB concernespecially in rural and underprivileged communities.

A significant discovery in this study is that 18.87% of MTB-positive instances displayed resistance to rifampicin, which is commonly seen as an indicator of MDR-TB. This ratio exceeds the global average of MDR/RR-TB in new cases, which is estimated to be around 3–5% (WHO, 2024). Nonetheless, comparable high rates have been documented in specific studies in Pakistan, particularly within previously treated or high-risk populations (Khan et al., 2022). The notable prevalence of rifampicin resistance observed in this study could be linked to inadequate treatment, poor adherence to therapy, and restricted availability of drug susceptibility testing

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services. These elements promote the development and distribution of resistant strains in the community. Analysis by age revealed that the largest share of MTB-positive cases was found in the 51–65 years age range, succeeded by those aged 36–50 years. This pattern indicates that TB mainly impacts those in economically active age brackets, potentially leading to significant socio-economic consequences. Comparable trends have been recorded in various regional investigations, where heightened exposure risk, job-related stress, and comorbidities like diabetes lead to elevated TB rates in middle-aged and older individuals (Riaz et al., 2023). While this study did not find a statistically significant link between age and rifampicin resistance, the distribution pattern underscores the necessity for specific screening strategies aimed at at-risk age groups.

Gender-based analysis indicated that men exhibited a greater prevalence of MTB and rifampicin resistance than women. This finding is consistent with global and national statistics, which consistently indicate a male predominance in TB cases (WHO, 2024). Potential reasons encompass increased exposure to environmental hazards, tobacco use, work-related risks, and variations in access to healthcare. In various circumstances, cultural and social obstacles might restrict women's access to prompt diagnosis, which can result in underreporting among females.

The distribution of cases by taluka further showed geographic differences in both MTB prevalence and rifampicin resistance. Khairpur taluka displayed the largest share of MTB-positive cases, whereas Kotdigi revealed the highest rate of rifampicin resistance. This variability may indicate differences in population density, healthcare systems, and public understanding of TB. These results emphasize the necessity of localized epidemiological monitoring to pinpoint high-risk locations and distribute resources efficiently.

The GeneXpert MTB/RIF assay employed in this study demonstrated its effectiveness as a diagnostic tool, facilitating quick identification of MTB and resistance to rifampicin. Molecular techniques like GeneXpert have greatly enhanced TB diagnosis by shortening turnaround time and boosting sensitivity, especially in smear-negative instances (Boehme et al., 2010; WHO, 2024). Relying only on rifampicin resistance as an indicator for MDR-TB might either underestimate or overestimate the actual burden, since resistance to isoniazid was not evaluated directly. Even with its advantages, the research has some limitations. The sample size was restricted to individuals visiting a single medical center, which may not completely reflect the larger population. Furthermore, the lack of comprehensive treatment history and molecular characterization of resistance mutations limits more profound epidemiological analysis. Future research should involve larger sample sizes, multi-center data, and sophisticated genomic methods to enhance understanding of transmission dynamics and resistance trends. In summary, the results of this research highlight the persistent challenge presented by TB and MDR-TB in Khairpur. Enhancing diagnostic capabilities, guaranteeing treatment compliance, and executing focused public health initiatives are vital to control the transmission of drug-resistant TB in the area.

5. Conclusion

This research offers valuable understanding of the impact of tuberculosis and multidrug-resistant tuberculosis (MDR-TB) in a high-risk rural area of Sindh, Pakistan. The results show that a significant number of suspected patients were diagnosed with Mycobacterium tuberculosis, and close to 20% of these cases showed rifampicin resistance, signifying MDR-TB. This emphasizes a troubling degree of drug resistance that surpasses global averages and highlights the ongoing difficulties in tuberculosis control locally. The prevalence of cases in economically active age groups and males indicates continued community transmission, likely affected by socio-economic conditions, delayed access to healthcare, and non-compliance with treatment. Even though a statistically significant relationship was not found between age and resistance patterns, the distribution trends are still epidemiologically important for specific interventions. In summary,

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the research emphasizes the critical necessity of enhancing TB control measures, such as prompt diagnosis using rapid molecular methods, better patient compliance with treatment, and broader drug susceptibility testing. Ongoing monitoring and localized data generation are crucial to guide public health strategies and lessen the spread of MDR-TB in marginalized communities.

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