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Determination of the Frequency of *Mycobacterium tuberculosis* Among 799 Suspected Tuberculosis Patients in the Population of District Okara, Punjab

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Abstract

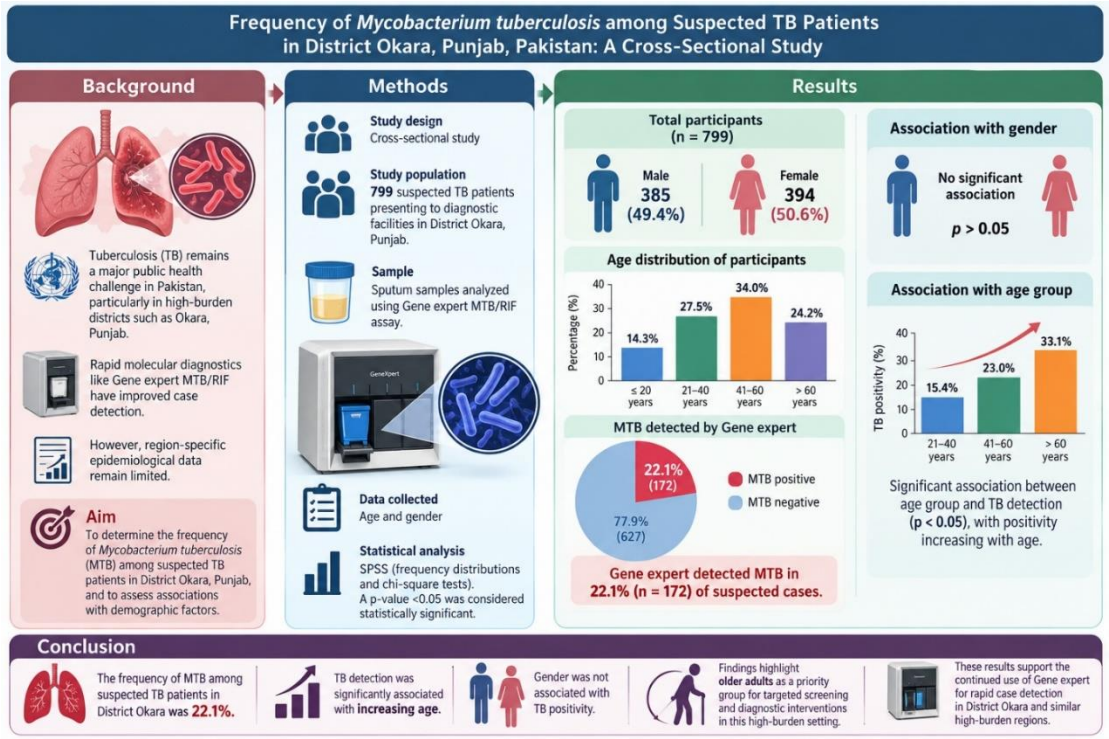
Background: Tuberculosis (TB) remains a major public health challenge in Pakistan, particularly in high-burden districts such as Okara, Punjab. Rapid molecular diagnostics like Gene expert MTB/RIF have improved case detection, yet region-specific epidemiological data remain limited. This study aimed to determine the frequency of *Mycobacterium tuberculosis* (MTB) among suspected TB patients in District Okara, Punjab, and to assess associations with demographic factors.

Methods: A cross-sectional study was conducted on 799 suspected tuberculosis patients presenting to diagnostic facilities in District Okara, Punjab. Sputum samples were analyzed using the Gene expert MTB/RIF assay. Demographic data including age and gender were recorded. Statistical analysis was performed using SPSS, with frequency distributions and chi-square tests applied to determine associations between demographic variables and TB detection. A p-value <0.05 was considered statistically significant.

Results: Among 799 participants, females constituted 50.6% (n=394) and males 49.4% (n=385). The 41–60 year age group was the largest (34.0%), followed by 21–40 years (27.5%). Gene expert detected MTB in 22.1% (n=172) of suspected cases. TB detection showed no statistically significant association with gender ($p > 0.05$). However, a significant association was observed between age group and TB detection ($p < 0.05$), with positivity increasing from 15.4% in the 21–40 year group to 33.1% among those aged >60 years.

Conclusion: The frequency of MTB among suspected TB patients in District Okara was 22.1%, with detection significantly associated with increasing age. Gender was not associated with TB positivity. These findings support the continued use of Gene expert for rapid case detection and highlight older adults as a priority group for targeted screening and diagnostic interventions in this high-burden setting.

Graphical Abstract



Introduction

Each year, TB remains one of the deadliest communicable diseases in the world, with an estimated 10.7 million cases and 1.23 million deaths worldwide in 2024, according to the World Health Organization (WHO). South-East Asia Region bears the world's highest burden, accounting for over one-third of incident cases while constituting less than one-quarter of the global population(Abayneh & Teressa, 2022). M. tuberculosis continues to strain diagnostic and treatment frameworks, especially in resource-limited settings where conventional diagnostic tools such as acid-fast bacilli (AFB) smear microscopy and solid culture continue to form the norm. Smear microscopy is fast and cheap, yet lacks sensitivity, especially in paucibacillary and paediatric disease, while culture, the so called 'reference' method, takes several weeks before results are obtained, further delaying therapy and infection control measures. Molecular platforms such as Gene expert MTB/RIF assay have transformed case detection, allowing simultaneous detection of M. tuberculosis complex DNA and rifampicin resistance in as little as 2 hours(Andarge et al., 2021). This innovation has found wide acceptance with the WHO and has been adopted into national tuberculosis control programs like the National Tuberculosis Elimination Programme of India, which recommends nucleic acid amplification testing as the primary diagnostic tool for all suspected cases of TB(Du et al., 2026). Against this background, the present study was conducted to determine the prevalence of M. tuberculosis among more than 500 patients with suspected TB using Gene expert platform, thus providing locally relevant epidemiological data to inform diagnostic and control strategies. Infections from diagnosis. As, we have previously described, the performance of the more advanced versions of the Gene expert as a diagnostic test for tuberculosis has been extensively analysed through meta-analyses(Girma et al., 2025). A large meta-analysis of 72 studies conducted by Wang et al. demonstrated that Ultra reaches a pooled sensitivity of 76% and specificity of 95% for pulmonary disease, still with better performance for rifampicin resistance (sensitivity 94%, specificity 97%), while a recent Cochrane review reported similar figures with a summary sensitivity and specificity of 90.7 and 94.8%, respectively, for pulmonary disease against culture(Harahap et al., 2026). The magnitude of performance based on the type of smear has also been highlighted, with a sensitivity of about 80.7% in smear-negative, culture-positive disease, exemplifying the challenge

that remains with paucibacillary disease. Moreover, in smear-negative pulmonary samples, 16.55% of cases of a negative microscopy resulted in expert MTB/RIF positive results, illustrating the applicability of the test also in smear-negative disease. Finally, as we saw above, Gene Xpert cycle threshold values have also been explored as an indirect measure of bacillary load, with various works suggesting low cycle threshold values are an indicator of more aggressive disease and could be used in conjunction with imaging to suggest phenotypes that could benefit from shorter drug regimens(Kassa et al., 2022). Epidemiology of detection of M. tuberculosis Noticeable geographical and demographic variation in the detection of M. tuberculosis reflects differences in tuberculosis prevalence and distribution, patient populations and operational capacity of diagnostic laboratories(Liu et al., 2026). A retrospective analysis of 9,468 clinical specimens submitted to a tertiary hospital in New Delhi revealed 10.45% positivity for M. tuberculosis, with pulmonary specimens presenting slightly higher yield (11.4%) than extra pulmonary specimens (9.5%). In a cohort of 153 patients evaluated for presumptive pulmonary tuberculosis in Sudan, 19.0% were identified as positive, with detection rates equivalent in fresh and processed samples; rifampicin resistance was identified in 3.4% of positive samples. Overall prevalence in 301 patients presenting with presumptive pulmonary disease in Ethiopia was 23.2%, with both age and clinical presentation influencing frequency(Sarkar & Sarkar, 2025). Diagnostic challenges are frequently compounded in pediatric populations with one study reporting positive detection of tuberculosis among 970 presumptive pediatric patients, with females having a significantly higher detection rate (44.4% versus 28.5%) and twice the risk of tuberculosis compared to males; adolescents (11-18 years) were more likely to be infected. These investigations emphasize how the detection of M. tuberculosis is governed by patient age, gender and specimen location along with national tuberculosis prevalence, reinforcing the need for continued surveillance(Wasihun et al., 2021). This is further complicated by the threat of multidrug resistant disease. A systematic review and meta-analysis of 102 studies and 4.12 million patients over 52 countries determined a notable decrease in the global prevalence of multidrug resistant tuberculosis/ rifampicin resistant tuberculosis between 2000-2009 (14.4%) and 2020-24 (2.6%), with a disproportionate burden in Africa (4.9%).2 Notably, the prevalence among previously treated patients remains high at 19.8% (26.2% in Africa), highlighting the need for diagnostic tools that readily identify the presence of disease and marker of resistance at the point of care(Aruga et al., 2025). In addition to improving accuracy and quantifying burden, subsequent studies have begun to focus more on the prognostic and therapeutic potential of Gene expert quantitative data. For instance, certain studies found that combined with clinical and radiographic information, the use of the expert MTB/RIF semi quantitative bacterial burden and severity of disease on chest x-ray successfully identified the 75% of patients in the RIFASHORT trial who experienced non inferior outcomes in a four-month rifampicin continuation regimen compared to the six-month standard, while a more difficult-to-treat phenotype of high bacterial burden and extensive disease accounted for over half of all relapses(Colichi et al., 2025). This conclusion has important implications for stratification and allocation of resources in high-burden countries. According to the WHO Global Tuberculosis Report 2025, there was a 2% decline in global incidence and a 3% decline in deaths from 2023 to 2024. Although 54% of cases were diagnosed with Gene expert in 2024 (up from 48% in 2023), this rapid diagnosis rate is still impeded by insufficient resources and the emergence of drug resistance. Additionally, incidence rates (201/100,000) in the WHO South-East Asia Region, which accounts for the largest absolute number of cases globally, have decreased by 16% since 2015 (barely above the global average), yet the region still suffers from the highest absolute number of cases globally, as well as stark divergences in incidence between countries. Poor nutrition, with nearly 850,000 cases of incident diabetes annually, has also been found to be a contributing factor in nearly all cases in

the South East Asia Region(Ge et al., 2026). In light of the heavy burden of disease, the limited resources available, and the challenge of the prevalence of both drug susceptible and resistant disease, accurate quantification of M. tuberculosis prevalence with molecular diagnostics is an immediate priority(Haider et al., 2025). The present study aims to fill this knowledge gap by providing empirical data on detection rates in a clinic population comprised of over 799 patients with clinically suspected tuberculosis, thereby contributing to the overall evidence base of molecular diagnostics for testing in similar high burden contexts(Glennon & Kroshinsky, 2025).

Materials and Method

Methods:

A cross sectional study was conducted on 799 suspected tuberculosis patients presenting to diagnostic facilities in District Okara, Punjab. Sputum samples were analyzed using the Gene expert MTB/RIF assay. Demographic data including age and gender were recorded. Statistical analysis was performed using SPSS, with frequency distributions and chi-square tests applied to determine associations between demographic variables and TB detection. A p-value <0.05 was considered statistically significant.

Results

Among 779 participants, females constituted 50.6% (394/779) and males 49.4% (385/779). The 41–60 year age group was the largest (34.0%), followed by 21–40 years (27.5%). Gene expert detected *M. tuberculosis* in 22.1% (172/779) of suspected cases. TB detection showed no significant association with gender ($p > 0.05$) but increased significantly with age, rising from 15.4% in the 21–40 group to 33.1% among those aged >60 years ($p < 0.05$).

Frequency Tables

Table 1: Gender Distribution

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Male	385	49.4	49.4	49.4
Female	394	50.6	50.6	100.0
Total	**779**	**100.0**	**100.0**	

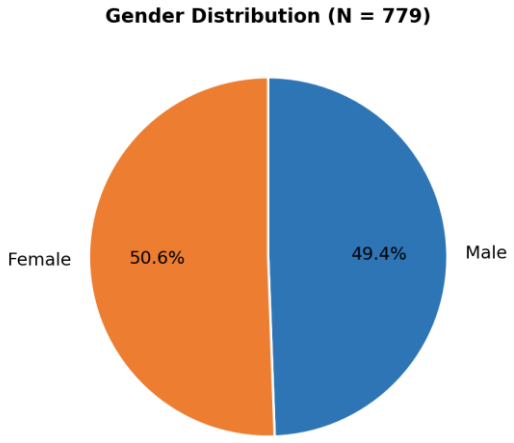


Figure 1: Pie chart showing gender distribution.

Out of 779 participants, 385 (49.4%) were male and 394 (50.6%) were female.

Table 2: Age Group Distribution

Age Group	Frequency	Percent	Valid Percent	Cumulative Percent
≤20 years	137	17.6	17.6	17.6
21–40 years	214	27.5	27.5	45.1
41–60 years	265	34.0	34.0	79.1

>60 years	163	20.9	20.9	100.0
Total	**779**	**100.0**	**100.0**	

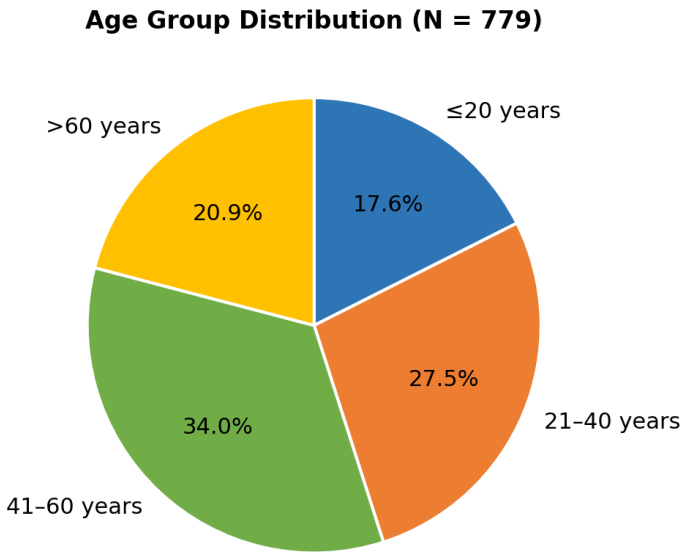


Figure 2: Pie chart showing age group distribution.

The largest group was 41–60 years (34%), followed by 21–40 years (27.5%).

Table 3: TB Test Result

TB Result	Frequency	Percent	Valid Percent	Cumulative Percent
Detected	172	22.1	22.1	22.1
Not Detected	607	77.9	77.9	100.0
Total	**779**	**100.0**	**100.0**	

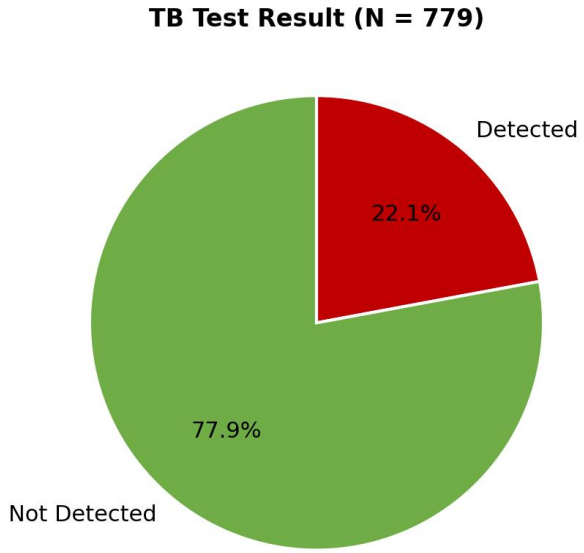


Figure 3: Pie chart showing TB detection.

TB was detected in 172 (22.1%) participants, while 607 (77.9%) were Not Detected.

3. Cross-tabulation (Chi-Square Test)

Table 4: Gender TB Result Cross tabulation

Gender	Detected n	Detected %	Not Detected n	Not Detected %	Total
Male	81	21.0	304	79.0	385
Female	91	23.1	303	76.9	394

Total	**172**	**22.1**	**607**	**77.9**	**779**

Table 5: Age Group TB Result Cross tabulation

Age Group	Detected n	Detected %	Not Detected n	Not Detected %	Total
≤20 years	24	17.5	113	82.5	137
21–40 years	33	15.4	181	84.6	214
41–60 years	61	23.0	204	77.0	265
>60 years	54	33.1	109	66.9	163
Total	**172**	**22.1**	**607**	**77.9**	**779**

There was a statistically significant association between age group and TB detection ($p < 0.05$). TB detection increased with age, from 15.4% in the 21–40 group to 33.1% in those above 60 years.

Discussion

In our current study, the overall detected proportion of MTB was 22.1% (172/779) in cases with suspected TB, which was comparable to the rates of 19.0% found in the Sudan study and 23.2% in the Ethiopian study but significantly higher than the detection rate of 10.45% reported in a tertiary care hospital in New Delhi, India. These differences might have been caused by the general burden of TB in the study areas, inclusion of sputum smear negative suspects, or presence of other patients' referral selection criteria(Nilsson et al., 2025). A report from Pakistan (Lahore) also using Gene expert in patients with cough, encountered a detection rate of 46.0% but in a smaller sample (50) and with inclusion of only patients with cough duration > 3 weeks, which would have enriched individuals with the most symptomatic disease and resulted in a higher proportion of detection than in our study . Our current study did not observe a sex difference in detection proportion, but this does not necessarily mean the same for the prevalence of TB. More importantly, this finding is consistent with our study population which also had no differences in sex prevalence(Pitakteerabundit et al., 2025). However, a systematic review reported that males are overrepresented at nearly 2-fold higher TB prevalence in low and middle income countries and have nearly 2-fold less utilization of care, which could be simply explained by lower access to care. This discrepancy might have been averted in our study because the study design was conducted in a clinical setting where symptomatic participants had to seek health care and underwent Gene expert testing. On the other hand, age was associated with risk of TB, which increased from 15.4% in the group of 21-40 years old to 33.1% in patients older than 60 years. These results are similar to what has been observed in the general population where the elderly have a higher prevalence of TB attributable to multiple factors including immune senescence, co-occurring diabetes, low body mass index and late presentation with symptoms(Wang et al., 2026) . The higher prevalence of TB in older patients increases the burden of disease in the South East Asia Region where more than 850,000 new TB cases were attributable to undernutrition in 2022, with an incidence rate of 201 per 100,000 population, clearly exceeding the global average of 131 per 100,000 population and the target of End TB globally. Reporting of rapid test coverage from 2023 to 2024 has been increased from 48% to 54%, according to the recent report by WHO on Global Tuberculosis Report 2025, there is an ongoing need for rapid test utilization in diagnosis of TB. The present study also confirms that Gene expert is an effective one-stop testing platform which can detect rifampicin resistance simultaneously while effectively detecting MTB from samples within 2 hours in resource limited circumstances with high disease burdens. The prevalence increase with age also indicates screening and rapid diagnostic approaches may need to be targeted on populations who are older, given the generally higher prevalence of TB(Yataka et al., 2026). Our finding of no sex based difference in detection rate should be interpreted with caution because symptomatic patients of both sexes will visit the health care places

in the current setting and may not reflect the epidemiology of the general population. In future investigation, history of previous treatments, comorbidities like diabetes and HIV status information should be supplemented to analyze prevalence in different demographic groups.

Integration with Other Sciences

Tuberculosis research increasingly requires an interdisciplinary approach integrating microbiology, molecular biology, clinical medicine, epidemiology, biomedical engineering, computational sciences, nutritional sciences, and public health. Such integration provides opportunities to improve the understanding of Mycobacterium tuberculosis (MTB), strengthen diagnostic interpretation, and develop more comprehensive approaches to disease surveillance and management.

Microbiology, Molecular Biology, and Diagnostic Sciences: Molecular biology and microbiological techniques provide the foundation for identifying infectious agents and investigating their biological characteristics. GeneXpert MTB/RIF represents an important application of molecular diagnostics because it enables the simultaneous detection of MTB and rifampicin resistance-associated markers. Research investigating the interpretation of GeneXpert results alongside sputum smear status further demonstrates the importance of integrating molecular and conventional microbiological findings when evaluating suspected pulmonary tuberculosis (Sarwar et al., 2026). Similarly, research on comparative HCV screening and confirmatory PCR highlights the broader importance of molecular confirmation and diagnostic accuracy in infectious disease testing (Nadeem et al., 2026).

Clinical Medicine, Pathology, and Epidemiology: Clinical medicine and epidemiology complement laboratory diagnostics by providing information about patient characteristics, disease presentation, and population-level detection patterns. The present study demonstrates this relationship through the identification of a 22.1% MTB detection rate among suspected TB patients and a statistically significant association between age and detection. Research investigating antimicrobial resistance and pathogen identification also highlights the importance of integrating laboratory findings with clinical assessment (Farrukh et al., 2026; Naik et al., 2026). Such multidisciplinary perspectives can support more comprehensive patient evaluation and epidemiological surveillance.

Biomedical Engineering and Medical Imaging: Biomedical engineering and medical imaging offer additional opportunities for investigating pulmonary diseases through image acquisition, processing, and computational interpretation. Research comparing handcrafted and convolutional features for multiclass lung cancer classification from CT images demonstrates the application of advanced image-analysis techniques in pulmonary disease assessment (Raoof et al., 2026). Although this investigation focused on lung cancer rather than tuberculosis, its methodological relevance suggests opportunities for future research evaluating computational imaging approaches in TB assessment, particularly through the integration of radiographic findings with molecular diagnostic results.

Artificial Intelligence and Computational Sciences: Artificial intelligence and computational modelling are increasingly relevant to the interpretation of complex biological and clinical information. Research on artificial intelligence-guided optimization of food fermentation demonstrates the application of computational approaches to microbial processes and process control (Khan et al., 2025). Additionally,

computational image classification research provides examples of machine-learning applications in medical image analysis (Raoof et al., 2026). These developments provide methodological perspectives for future TB-specific investigations exploring the integration of molecular results, clinical characteristics, and imaging information. Nevertheless, their diagnostic value in tuberculosis requires independent clinical validation.

Nutritional Sciences and Host–Microbiome Interactions: Nutritional sciences provide another relevant perspective for understanding the broader biological factors associated with human health and immune function. Research investigating gut microbiota modulation through functional foods has examined relationships between microbial communities and low-grade chronic inflammation (Nawaz et al., 2026). Similarly, research on plant-based functional foods has explored their nutritional potential and health implications (Riaz et al., 2026). These studies provide broader biological context for investigating nutrition, inflammation, and host health. However, the specific contribution of nutritional status and microbiome alterations to MTB detection and tuberculosis outcomes remains an area requiring dedicated investigation.

Environmental Sciences and Molecular Surveillance: Environmental sciences and molecular surveillance provide complementary approaches for understanding microbial distribution and environmental exposure. Environmental DNA-based detection has demonstrated the application of molecular techniques for biodiversity monitoring across freshwater and terrestrial ecosystems (Iqbal et al., 2024). Furthermore, research investigating emerging environmental contaminants in aquatic organisms illustrates the application of molecular and toxicological approaches to environmental health assessment (Ullah et al., 2026). Although these studies do not directly investigate MTB transmission, they demonstrate the wider applicability of molecular surveillance techniques and provide a conceptual basis for exploring environmental determinants of infectious disease epidemiology.

Pharmaceutical Sciences and Antimicrobial Resistance: Pharmaceutical sciences and antimicrobial resistance research are important components of comprehensive tuberculosis management. The simultaneous identification of MTB and rifampicin resistance through GeneXpert provides a direct connection between molecular diagnosis and antimicrobial treatment decision-making. Broader investigations into antimicrobial resistance mechanisms and laboratory detection approaches further emphasize the importance of reliable resistance identification (Naik et al., 2026). Research on the synthesis of functionalized organic molecules also demonstrates developments in chemical synthesis relevant to pharmaceutical and industrial applications (Raza et al., 2026). However, the therapeutic effectiveness of such compounds against MTB has not been established by these references.

Future Perspectives

Future investigations should expand the epidemiological assessment of tuberculosis through larger, multicenter, and population-based studies involving diverse geographical regions of Punjab and other high-burden areas of Pakistan. Although the present study identified a substantial MTB detection rate among suspected tuberculosis patients in District Okara, its cross-sectional design and clinical-based sampling limit the generalizability of these findings to the wider population. Further research should incorporate longitudinal surveillance to investigate variations in disease detection, identify emerging epidemiological patterns, and evaluate changes in tuberculosis burden over time.

An important direction for future research is the comprehensive evaluation of molecular diagnostic performance through comparisons between GeneXpert MTB/RIF, GeneXpert MTB/RIF Ultra, sputum smear microscopy, and mycobacterial culture. Particular attention should be directed toward smear-negative and paucibacillary tuberculosis cases, where conventional diagnostic approaches may fail to identify infection. Furthermore, investigations examining the relationship between GeneXpert results, semi-quantitative bacterial load, and sputum smear status could improve the interpretation of molecular diagnostic findings and support more comprehensive patient assessments (Sarwar et al., 2026). Comparative diagnostic research using molecular confirmation and PCR-based approaches may also provide useful methodological insights for evaluating diagnostic sensitivity, specificity, and discordant results in infectious disease testing (Nadeem et al., 2026).

Future studies should also incorporate additional clinical and epidemiological variables, including previous tuberculosis treatment, diabetes mellitus, HIV status, nutritional status, smoking history, and socioeconomic characteristics. These factors may help explain variations in MTB detection across different demographic groups and identify populations requiring more focused diagnostic interventions. Given the significantly higher detection rate observed among individuals older than 60 years in the present study, prospective investigations should particularly examine the contribution of age-related immune changes, comorbidities, and delayed healthcare-seeking behavior to tuberculosis detection in older adults.

Another important research priority is the systematic investigation of rifampicin resistance and other forms of drug-resistant tuberculosis. Although GeneXpert MTB/RIF provides simultaneous detection of MTB and rifampicin resistance, future studies should establish the prevalence and distribution of resistance among molecularly confirmed cases and incorporate complementary drug susceptibility testing where appropriate. Advances in laboratory-based antimicrobial resistance detection, including emerging molecular approaches, may provide additional opportunities to strengthen resistance surveillance and improve diagnostic decision-making (Naik et al., 2026).

Furthermore, emerging computational approaches, including artificial intelligence and machine learning, may offer opportunities to integrate molecular diagnostic findings with clinical information, sputum characteristics, and chest radiographic features. Research exploring computational analysis of respiratory imaging has demonstrated the potential of advanced feature extraction and classification techniques in pulmonary disease assessment (Raoof et al., 2026). Future TB-specific investigations could evaluate whether these approaches improve risk stratification, diagnostic interpretation, and identification of individuals requiring confirmatory testing. However, their clinical utility would require independent validation using appropriately characterized tuberculosis datasets.

Finally, future research should evaluate the feasibility, affordability, and accessibility of rapid molecular diagnostic platforms in district-level healthcare facilities, particularly in resource-constrained settings. Integrating molecular testing with targeted screening, improved referral systems, and continuous epidemiological monitoring may facilitate earlier diagnosis and timely treatment initiation. Collectively, these research directions could strengthen the evidence base for tuberculosis surveillance in Pakistan and contribute to more effective, evidence-based strategies for achieving national and global tuberculosis elimination targets.

Conclusion

Gene expert detected *M. tuberculosis* in 22.1% of suspected TB patients, confirming a substantial diagnostic yield in this population. TB detection was significantly associated with increasing age, with the highest positivity among those over 60 years. Gender was not associated with detection, suggesting equitable access among symptomatic patients undergoing molecular testing. These findings support the

continued deployment of Gene expert for rapid case detection and highlight older adults as a priority group for intensified screening.

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