

DIAGNOSTIC PERFORMANCE OF MOLECULAR VERSUS CONVENTIONAL TB DETECTION METHODS IN A RESOURCE-LIMITED DISTRICT OF AZAD JAMMU AND KASHMIR

Muhammad Soaib Said^{*1}, Nayab Imtiaz², Zarish Altaf³, Mishal Azhar⁴, Noor-ul-Eman⁵, Hina Aftab⁶, Muniba Rehman⁷, Izhar Ullah⁸, Iqra Saleem Naz Babari⁹, Kashif Sohail¹⁰, Tahira Batool¹¹

^{*1,10}Department of Pharmacy, Women University of AJ & K Bagh AJK

^{2,3,4,5,6,7}Department of Allied Health Sciences, Women University of AJ & K Bagh AJK

^{8,9}Department of Pharmacy, University of Poonch Rawalakot AJK

¹¹Department of Biotechnology, Women University of AJ & K Bagh AJK

^{*1}soaib_said@yahoo.com

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Corresponding Author: *

Muhammad Soaib Said

Abstract

Tuberculosis (TB) is also one of the major health problems facing the world especially in developing countries like Pakistan. The disease still kills millions of people every year despite decades of campaigns by the entire population about the disease. The proposed study is expected to assess the diagnostic tools of tuberculosis in the District Headquarters (DHQ) Hospital Bagh Azad Jammu and Kashmir (AJK), and assess whether the Directly Observed Treatment Short-Course (DOTS) program has become effective in the same environment. A retrospective, cross-sectional, and descriptive study was done in 100 patients suspected of TB at DHQ Hospital Bagh. Diagnostic techniques such as the use of Ziehl-Neelsen (ZN) smear microscopy, GeneXpert MTB/RIF testing, and chest radiography were compared in their sensitivity and diagnostic sensitivity of the test. Patient compliance and outcomes of treatment were also assessed at the framework of the DOTS. The results showed that 26 of the patients met the positive test by ZN smear microscopy, and 29 had the positive test by GeneXpert MTB/RIF, meaning that molecular diagnostics are more sensitive. In 38 percent of cases, Chest X-rays were positive of TB. Feedback on treatment outcome indicated that the DOTS program had a 78 percentage completion and 22 percentage default. The findings indicate that there are diagnostic and treatment difficulties that are based on resource constraints, socioeconomic limitations, and geographical obstacles that are common in AJK. It is established in the study that effective TB control in rural and mountainous areas such as AJK requires the empowerment of molecular diagnostics, enhanced patient follow-up systems, and community-based DOTS. The results can add value to the Pakistan End TB Roadmap 2030 with its focus on patient-centered strategies and decentralization of diagnostics to realize a more successful TB control district-level result.

INTRODUCTION

Tuberculosis (TB) remains among the most endemic infectious diseases in the world, causing significant threats to the general public health as well as being preventable and curable. Mycobacterium tuberculosis

is the cause of the disease and it is one of the main causes of death in the whole world even in annuities exceeding HIV/AIDS and malaria [1]. The World Health Organization (WHO) states that there were

almost 10.8 million new TB infections and 1.25 million deaths in 2023, which requires immediate diagnostic and treatment interventions [2]. The low and middle-income countries are the main burden of TB, in which poverty, mal-nutrition, overcrowding, and insufficient healthcare facilities provide good grounds where the disease spreads [3]. Pakistan is the fifth most populous country in the South/Southeast Asia and it has a high burden of TB and it is contributing to the global incidence. According to national reports, the estimated cases of 250 per 100,000 populations with a lot of difference between provinces and regions [7]. AJK is of particular concern as it is a mountainous area, with a sparse rural population and a scarce number of diagnostic centers, which prevents early diagnosis and lifelong treatment [7b]. The COVID-19 pandemic has additionally impacted the TB diagnostic and treatment service and caused delays in the detection of cases and initiation of treatment all over the world [2]. In their turn, WHO has stressed the need of integrating the molecular testing systems like GeneXpert MTB/RIF and GeneXpert Ultra to improve the quality of diagnosis and resistance to the drug monitoring in the high-burden environments [6]. Nevertheless, there are still issues of implementation such as lack of proper infrastructure, unstable power supply and the high cost of operation particularly in rural hospitals such as DHQ Hospital Bagh [6a]. Under these limitations, this research paper investigates the diagnostic practices in use at DHQ hospital bagh, AJK and also analyzes the effectiveness of the Directly Observed treatment short-course (DOTS) program. The study offers a regionalized insight into the issue of TB control and fits the Pakistan End TB Roadmap 2030, which suggests patient-centered care and better diagnostic coverage.

METHODOLOGY

STUDY DESIGN

The research design of this study was the descriptive, cross-sectional, and retrospective observational design to assess the strategies of diagnosis of tuberculosis (TB) and the effectiveness of the Directly Observed Treatment Short-Course (DOTS) program in the District Headquarters (DHQ) Hospital Bagh, Azar Jammu and Kashmir (AJK). The methodology used in the study was both quantitative data of patient records and laboratory registries and qualitative data of structured patient interviews. The design is concordant with the operational research principles of WHO that focus on the evidence-based assessment of

national TB control operations to enhance the local implementation strategies [17]. The descriptive part was to establish the frequency, distribution and performance of diagnostic techniques, such as Ziehl-Neelsen (ZN) smear microscopy, GeneXpert MTB/RIF assay, and chest radiography. The retrospective element was reviewing patient and laboratory records, whereas the cross-sectional model allowed assessing the effectiveness of the diagnosis and the compliance with treatment at the same time during the study.

STUDY SETTING

The research was done at DHQ Hospital Bagh which is the main tertiary care hospital in Bagh district of AJK that serves a population of more than 500000 people. The hospital is part of the Common Management Unit (CMU) of the National TB Control Programme (NTP) in Pakistan and offers diagnostic services to customers such as microscopy, radiography and molecular testing. It has a GeneXpert system, Ziehl-Neelsen staining plant and a digital X-ray unit in its hospital. Nevertheless, frequent power failure, low labour and lack of technical training inhibits the optimal use of these resources [14]. The mountainous nature and rural population of the Bagh district pose a major logistical problem in healthcare provision. TB diagnostic and treatment services are accessible in the inner part of the city to many patients who live in the surrounding areas (711 patients within 20-40 kilometer radius). As a result, many patients take lack of discovery, long disease diagnosis and inconsistent medication adherence. These characteristics make DHQ Bagh a good location to assess the effectiveness of interventions on TB control within a resource limited setting [23]

POPULATION AND SAMPLING OF THE STUDY.

A population of 100 patients visiting the DHQ Hospital Bagh TB clinic or outpatient department during the period May to September 2025 were the members of study population. Clinical signs and symptoms were observed in all of the participants and were indicative of pulmonary or extra-pulmonary TB such as persistent cough (over two weeks), fever, night sweats, weight loss, or hemoptysis. It employed a non-probability consecutive sampling method in which all eligible patients between the periods of the study were employed until the target sample size was achieved. This sampling method reduced the selection

bias and provided the actual load of TB cases in the hospital. Patients whose anti-TB treatment history exceeds one month as well as those with incomplete diagnostic information were excluded. The presence of male and female patients made it possible to compare the trends in the diagnostic and treatments of the patients with respect to gender [17]

DATA COLLECTION PROCEDURE

The data was collected in two steps, which were retrospective record review and prospective patient interview

Phase I: RETROSPECTIVE RECORD REVIEW

- Laboratory registers, treatment cards on patients and cohort records were used to retrieve data stored under NTP system.
- The District TB Information System (DTIS) was used to confirm the diagnostic results, demographic, and treatment outcomes.
- In a bid to make sure that the data collected is accurate, data collectors were taken through three-day training session to learn how to abstract and control quality of records under supervision of the district TB coordinator.

Phase II: PATIENT INTERVIEWS

- Thirty patients were interviewed in a structured manner to determine their perception regarding the accessibility of diagnostics, stigma, and barriers to treatment adherence.
- The interviews were conducted either in Urdu or Pahari, as desired by the patient, and they were guided by the WHO People-Centered TB Care Framework (2020).
- All participants were informed of their rights to participate in interviews by giving verbal and written consent [11]

DIAGNOSTIC PROCEDURE

Three modalities on diagnosis were studied on the basis of performance, turnaround time and sensitivity:

ZIEHL-NEELSEN (ZN) SMEAR MICROSCOPY

Each patient was sampled (two sputum samples) in spot and early morning. The stains of smears were done by Ziehl-Neelsen method and observed under oil immersion microscopy with regards to Acid-Fast Bacilli (AFB). The grading of results was based on NTP criteria of scanty, 1+, 2+ and 3+. All efforts to ensure

quality were put in place, external Quality Assurance (EQA) was done every quarter, 10 percent of negative and 100 percent of positive smears were re-examined by the Provincial TB Reference Laboratory, Muzaffarabad.

CHEST RADIOGRAPHY

The chest X-ray was done in all presumptive TB cases. Radiographs were classified as either suggestive, indeterminate or normal. Inter-reader reliability was determined by having a second radiologist carry out a review of 20% of the films on their own. This technique was invaluable in diagnosing essential cases where grouping could not be done through bacteriological confirmation.

MOLECULAR TESTING

The molecular testing was done on GeneXpert Ultra using the WHO standard operating procedures (SOPs, 2021). The results were categorized in either of the following categories: MTB detected, MTB not detected, and invalid/error. The test raised the simultaneous rifampicin resistance which is a rapid clue of possible multidrug-resistant-TB (MDR-TB). The turnaround time was monitored and missed runs were recorded in order to measure the efficiency of operations.

DATA ANALYSIS AND DATA MANAGEMENT

The data were keyed into the SPSS version 26 and were triangulated by two researchers to facilitate consistency. Frequencies and proportions have been used to analyze categorical variables (ex: sex, test type, treatment outcome), whereas means $\pm$ standard deviation (SD) have been used to describe continuous variables (ex: age, diagnostic delay).

Analytical tests

- **Diagnostic yield analysis:** The rate of positive cases that each of the test modalities identifies.
- **Concordance analysis:** Cohens Kappa was employed to determine the level of agreement between results of ZN microscopy and GeneXpert.
- **Predictive modeling:** A logistic regression was used to determine demographic and clinical predictors of bacteriological confirmation.

RESULTS

The paper represents the results of 100 tuberculosis (TB) patients diagnosed and treated in the District Headquarters (DHQ) Hospital Bagh, Azar Jammu and Kashmir (AJK). The clinical records, lab reports, and treatment logs gathered during the period of the National TB Control Programme (NTP) (May and September 2025) were used to compile data. The SPSS version 26 was used to conduct statistical analysis and the results included the diagnostic yield, treatment outcome, and cross-comparative performance of the different testing modal. The population of the study will consist of a population stratified by gender and health status. 3.1 Summative of Study Population The study population will be a stratified population based on gender and health status. Out of 100 children incorporated in the study, the age group was between 18-70 years (mean age 39.5 +/- 11.2 years). The female to male ratio was 1.3:1 which showed that there is a minor prevalence of TB among males. In the rural regions, the proportion of patients was about 68 percent and it was 32 percent in the urban region. The socioeconomic stratification indicated that the patients were mostly in low-income households (72%). These data indicate the demographic features of AJK where the limited resources and the geographical location impact considerably on the access to healthcare [7b]. The majority of patients were reported to have persistent cough (95%), fever (84%), and weight loss (63%) and hemoptysis in 28% of the cases. This fact, that the proportion of pulmonary symptoms is high, demonstrates the prevalence of pulmonary TB in

the study population in agreement with the regional and global epidemiological data [1][3].

DIAGNOSTIC YIELD OF ZIEHL-NEELEN (ZN) SMEAR MICROSCOPY

It was found that 26 of the 100 samples analyzed showed a positive result using the Ziehl-Neelsen (ZN) smear microscopy (26%). The rest 74% were smear negative. These percentages are consistent with the operational research results in Pakistan whereby, ZN smear positivity among presumptive TB cases is usually between 20-30 The low positivity rate is associated with a number of operational and biological factors, such as poor quality of sputum, late health-seeking pattern, and extrapulmonary or paucibacillary disease. Moreover, malnutrition and HIV co-infection are also co- morbidities that have been known to decrease the sensitivity of the smear microscopy because of low bacteria load [4]. In spite of these shortcomings, ZN microscopy is an important first-line diagnostic tool in resource-limited environments because it is cheap and has a short turnaround time. Accuracy and reliability was ensured by regular External Quality Assurance (EQA) tests, held by the Provincial TB Reference Laboratory in Muzaffarabad. The discrepancies in microscopy and molecular technologies observed however, emphasize the importance of integrating the use of GeneXpert testing as a confirmatory diagnostic test [5][6].

Table 1: Diagnostic Yield of ZN Smear Microscopy (N = 100)

Category	Count (out of 100)	Percentage	Context/Note
Smear Positive	26	26%	Consistent with operational research in Pakistan (20–30% positivity among presumptive TB cases).
Smear Negative	74	74%	
Total Samples	100	100%	Overall diagnostic distribution based on ZN smear microscopy.

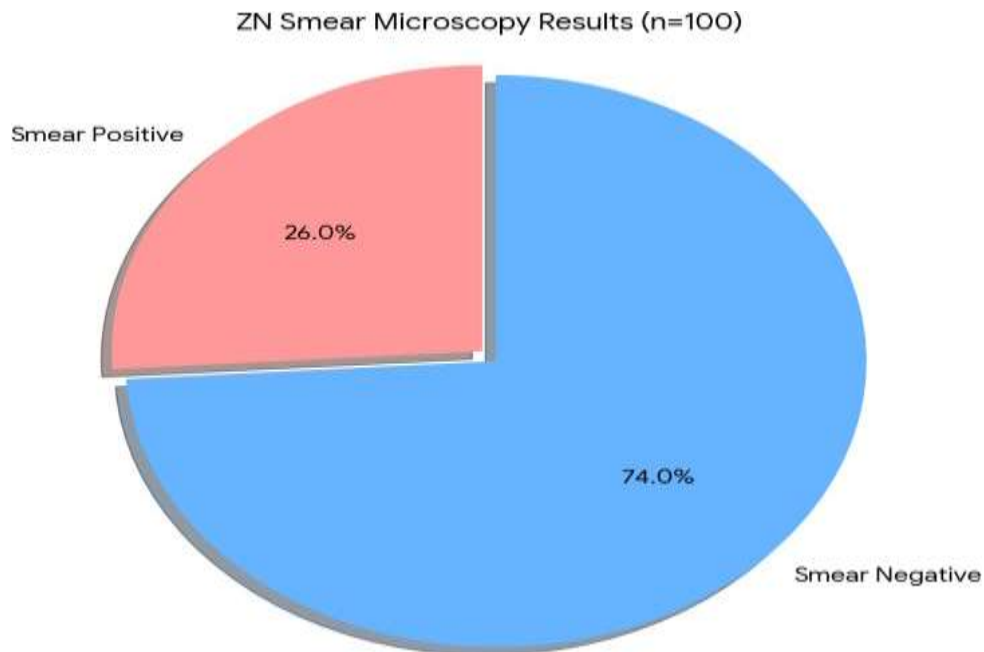


Figure 1: DIAGNOSTIC YIELD OF ZIEHL-NEELSEN (ZN) SMEAR MICROSCOPY

GENEXPERT MTB/RIF ASSAYS

GeneXpert MTB/RIF test identified Mycobacterium tuberculosis in 29 of them (29%), and 71 were negative. This dataset had no resistance to rifampicin. This molecular test was found to be more sensitive than the ZN microscopy with three additional TB positive cases not detected by the smear analysis.

This finding is in line with the WHO data showing that molecular diagnostic tests like GeneXpert can identify up to 30-35% more cases than smear microscopy, especially in smear negative or patients who are co-infected with HIV [2][6]. GeneXpert is a better diagnostic tool because it can detect rifampicin resistance at the same time (in less than 2 hours) and it has a better turnaround time. The GeneXpert system of DHQ Hospital Bagh had been experiencing occasional power disconnection and

shortage of cartridge; hence occasional delays. Test results could be obtained in 48-72 hours on average, which was better than the recommended 24 hours by the WHO [6a]. In this sort of resource-constrained districts the growth of solar-powered systems and better supply logistics would help greatly to improve efficiency.

The results of the GeneXpert support the centrality of the test in the realization of early and correct

identification of TB, particularly when it is incorporated with other microscopy-based screening. The research notes that the state needs long-term financial contribution and technological assistance to have continuous capacity to conduct molecular tests in AJK [12][14].

Table 2: TB Positive Cases Detected by Diagnostic Method

Diagnostic Method Detected	TB Positive Cases
Microscopy	26 cases (29 - 3)

GeneXpert
MTB/RIF

29 cases
ZN

Smear

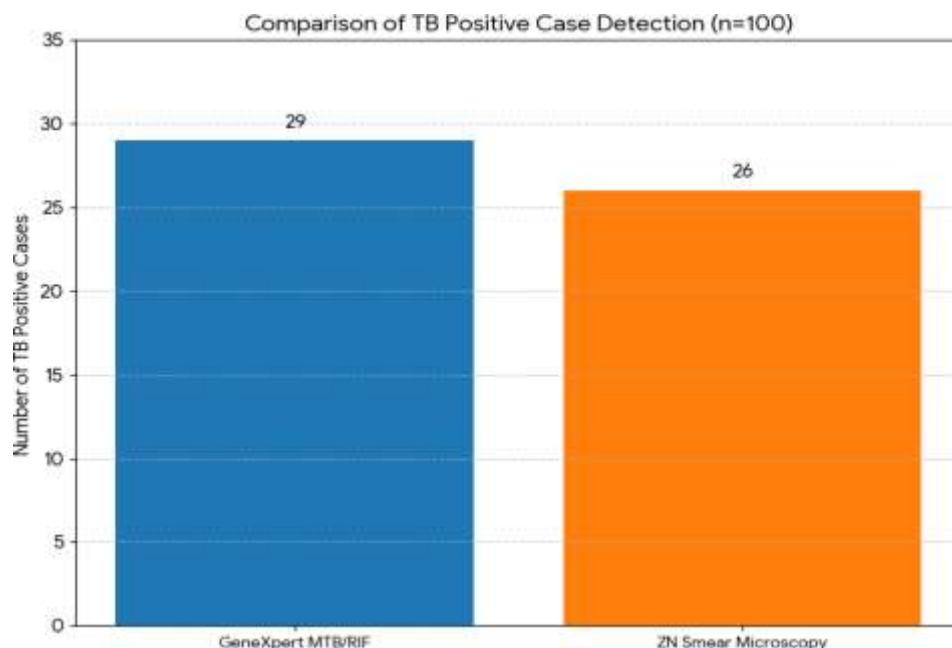


Figure 2: Comparison of GeneXpert and ZN Microscopy Results

CHEST RADIOGRAPHY FINDINGS

Among the 100 patients that were studied, 38 percent exhibited radiographic features indicating active tuberculosis and 62 percent of the cases had normal to non-specific lung appearances. The typical radiographic results were upper-lobe infiltrates, cavities and nodular densities. These findings are in line with national operational research studies that show that 35-40% of presumptive TB patients have radiographic findings that suggest disease [10]. Nevertheless, the active TB cannot be diagnosed by X-rays of the chest only, because the same anomalies can also be observed in such conditions as bronchitis (chronic) or pneumonia. The variable aspect of radiographic interpretation can be a weakness especially in non-central hospitals with low expertise in radiology. However, in such environments as DHQ Bagh where molecular tools are not necessarily present, chest X-rays can still serve as an effective and indispensable tool of adjunctive diagnosis to detect and make a clinical decision early in the development of the disease [7][11][24]. The radiographically normal rates (62) could be a result of timely detection of the disease or non-TB respiratory diseases. It further emphasizes the need to combine radiography with bacteriology and molecular tests in order to manage the case in full.

EFFECTS OF TREATMENT UNDER DOTS PROGRAM

The results of treatment using the Directly Observed Treatment Short-Course (DOTS) program showed that 78 percent of patients completed the treatment program, and 22 percent did not adhere to therapy or discontinued it. These results are quite similar to the national TB performance indicators, where the rates of treatment success usually lie between 80-85, and the rate of default between 15-20 [7][9]. This is high completion rate which indicates the high functioning capacity of the DOTS framework at DHQ Bagh that is facilitated by the constant supply of drugs and monitoring of adherence. Nevertheless, the 22% default rate means that the problem of socioeconomic hardship, travel distance, and social stigma still persists. Remote village patients have been found to experience challenges in commuting every day or every week to receive supervised care especially women who are bound by cultural standards [10][14]. The community-based DOTS (CB-DOTS) programs have been making some headway in resolving these obstacles. The implementation of Lady Health Workers (LHWs) and trained community volunteers to monitor treatment in the homes of patients in such districts boosted treatment adherence to almost 90% by 2023 [10]. Nevertheless, lack of capacity to staff and training is still an issue to mass CB-DOTS in AJK.

The agreement between diagnostic tests is assessed based on the results obtained from the tests on excitement, anxiety, and depressive disorder.

CONCORDANCE OF DIAGNOSTIC TESTS

The concordance of diagnostic tests is determined according to the findings of the diagnostic tests of excitement, anxiety, and depressive disorder. When comparing the outcomes of ZN microscopy and GeneXpert, there were moderate diagnostic concordance rates of both tests as 16 patients had positive results and 61 patients had negative results. A total of thirteen GeneXpert-positive patients were ZN-negative, indicating the better sensitivity of GeneXpert to low loads of bacteria.

DISCUSSION

DISCUSSION OF SIGNIFICANT RESULTS

The current research presents an overall assessment of diagnosis and treatment outcomes of tuberculosis (TB) in DHQ Hospital Bagh, Azad Jammu and Kashmir (AJK). The results highlight the relevance of combining old and new diagnostic methods such as the Ziehl-Neelsen (ZN) microscopy, GeneXpert MTB/RIF, and chest radiography in the precise identification of TB cases in a resource-constrained environment.

The above 29% GeneXpert positive against 26% smear positive indicate that molecular diagnostics is a serious constructive of the detection of TB, particularly in smear negative patients. This is in line with the recommendations of the WHO, which recommends GeneXpert as a diagnostic test of first line in areas of high burden [2][6]. The intermediate correlation (Cohen k) between ZN microscopy and GeneXpert indicates that although smear microscopy is still a helpful screening method, it is not a tool that can be reliably used on its own when making a diagnosis. Molecular testing has come to the rescue of this diagnostic gap by identifying the cases with low bacillary load that microscopy may not be able to detect [5][17]. Also, the 38% radiographic evidence of TB supplements the findings of laboratory tests, and the importance of the use of chest X-rays as an essential additional diagnostic test is demonstrated in cases when the results of the bacteriological examination are inconclusive. This is a unified method of action, and it helps to enhance the recommendation of WHO, who recommends combined diagnostic approaches to enhance early detection and minimise disease transmission [12]. The

high treatment completion rate of 78% with the Directly Observed Treatment Short-Course (DOTS) program is a sign of high efficiency in its operations at DHQ Bagh, but the default rate of 22% is a constant reminder of the socioeconomic barriers, stigma, and the logistics issues affecting success. These results are in line with national reports that indicate 80-85% success rates of treatment and 15-20% default rate in such settings [7][9].

DIAGNOSTIC EFFICIENCY AND PROBLEMS

Although ZN microscopy is an affordable and easily available screening test, it has low diagnostic sensitivity particularly in extrapulmonary or paucibacillary TB [4][5]. The findings of this research prove that the use of smear microscopy alone may lead to under-diagnosis and delay in the initiation of treatment. A more sensitive, specific, and rapid detection of an extra 3% of this cohort is possible with the use of molecular testing with GeneXpert MTB/RIF. Nevertheless, there are various limitations to the operations that can be experienced when using the GeneXpert in a rural hospital like the DHQ Bagh. These are recurrent power failure, shortage of cartridges, and low technical training which results in turn around times that are above the recommended 24-hour limit by WHO [6a]. In spite of these constraints, GeneXpert has helped increase diagnostic accuracy, make it easier to detect rifampicin resistance early and tailor treatment regimens in the TB control program in Pakistan more precisely [10][14]. Although non-specific, chest radiography is one of the useful adjuncts, especially in the absence of molecular testing. A total of 38 percent of patients in this study showed radiographic changes that were suggestive of TB, which makes it important in the context of case screening and clinical triage. Nevertheless, variability in interpretation and lack of radiographic services particularly of a female patient due to the cultural factors are significant issues [7b].

PROGRAMMATIC IMPLICATIONS AND TREATMENT OUT-COMES

The high level of treatment completion to 78 percent by the DOTS program shows the efficacy of the supervised treatment in enhancing treatment compliance and lowering the rates of relapse. Nevertheless, the 22 percent rate of treatment defaults that were found in this research is a major issue. Among the factors associated with non-adherence,

long travel distance, economic factors, gender mobility restrictions and social stigma against TB have been identified [9][10]. These findings resonate with other studies done in the region and report challenges similar to the mountainous and rural communities. In AJK, several patients have to travel 20-40 kilometers to the DHQ Hospital leading to inconsistent follow-ups and missed doses [14]. Especially women have limitations in mobility and are stigmatized by the society, which is likely to make them stop treatment. The use of Community-Based DOTS (CB-DOTS) programs has been proven to be a possible solution to those issues. CB-DOTS has enhanced success rate of 80% to 90% by involving Lady Health Workers (LHWs) and community volunteers to deliver drugs and supervise their compliance in a number of AJK districts [10]. Patient monitoring can be further enhanced with the incorporation of digital adherence technologies (mobile-based reminders (99DOTS)) and video-observed therapy (VOT) which can help decrease stigma due to the ability to conduct monitoring remotely [27]. Such community-based innovations are compatible with the objectives of the WHO End TB Strategy (2030) and the national TB Control Programme (NTP) in Pakistan, which focus on patient-centered care, decentralized diagnostics, and online monitoring to enhance the outcome of the treatment process [2][12][18].

COMPARISON TO THE PAST RESEARCH

This study has found similar results with the national and international studies that indicate the complementary nature of the molecular and standard diagnostic tools. According to the studies of Punjab and Sindh provinces, GeneXpert positivity is found to be between 25-35, which proves its greater sensitivity than the smear microscopy [7][19]. In the same vein, studies have been done in other South Asian nations like India and Nepal and have demonstrated that the combination of GeneXpert with routine microscopy enhances the detection of the cases by up to 30 percent and the diagnostic delays [20][26].

When it comes to treatment outcomes, the 78% completion rate is indicative of an equivalent result of operational surveys in Khyber Pakhtunkhwa and Punjab where treatment success rates fell within the range of 75-85% in the framework of DOTS [6][13]. The 20-25 percent default rate, however, has been persistent and is a common issue in a rural health system in South Asia.

PUBLIC HEALTH AND POLICY IMPLICATIONS

The results of the study have a strong policy implication in TB control in Pakistan (as well as in low-resource countries). First, the findings suggest greater GeneXpert extension and foster better laboratory infrastructure in the hospitals on the district level. The deployment of solar backup, the reinforcement of the sample transportation systems, and the frequent technical training of the laboratory personnel can make a significant contribution to the effectiveness of diagnostics [14][25].

Second, the high treatment default rate demands an improved community involvement and patient-based support systems. Travel subsidies, nutrition and counseling especially to women and low-income patients can enhance adherence and outcomes. The use of digital tools such as mobile health apps and automated adherence reminders can also transform the DOTS implementation and decrease stigma around TB treatment [10][27].

CONCLUSION

This paper was carried out in DHQ Hospital Bagh, Azarajammu and Kashmir, to give a critical analysis of tuberculosis (TB) case-finding techniques and effectiveness of the Directly Observed Treatment Short-Course (DOTS) program in a resource-poor, mountainous area. The results underline that although the world has made significant progress in TB diagnosis and treatment, poverty in rural areas of Pakistan in terms of inadequate healthcare, socioeconomic factors, and logistical problems remain as challenges to effective TB management in rural Pakistan. Comparison of diagnostic tools demonstrated that the GeneXpert MTB/RIF was better than Ziehl Neelsen (ZN) microscopy with smears in sensitivity and accuracy. Molecular diagnostics increased the total detection rate and early detection of patients with TB-positive condition even those with low bacterial load. Chest radiography was also found useful as an additional diagnostic measure particularly where there were inconclusive results on bacteriology. These methods of diagnosis combine to create a support system that enhances the accuracy and promptness of the TB diagnosis in under-resource healthcare facilities.

The result of the analysis of the treatment outcomes indicated that the success rate under the DOTS program was 78 percent, which proves the strength of the program operation and its dedication of the

medical personnel of the DHQ Hospital Bagh. Nonetheless, the default rate is 22% which means that patient compliance is still a significant issue that is affected by the barriers to travel, stigmatization, gender, and economic difficulties. By involving communities, conducting patient education, and decentralization of service delivery, these issues can be addressed to a great extent and improve the effectiveness of the program.

The research highlights the necessity to invest in diagnostic infrastructure, maintain continuous power supply and access to the tests, and provide molecular testing to the peripheral health centers. Moreover, community-based DOTT activities with the assistance of digital adherence technologies may guarantee the improvement of the patient monitoring process and the success of the treatment.

To sum up, tuberculosis is one of the primary public health problems in Azad Jammu and Kashmir. This research study has offered a localized model that may be applied in other rural districts in Pakistan as a way of improving the overall effectiveness and sustainability of the national TB control strategy. elimination goals.

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