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RESEARCH ARTICLE

Molecular Diagnostics for Tuberculosis: Gene Xpert MTBC/RIF Assay for Rapid Detection of Mycobacterium Tuberculosis and Extensively Drug-Resistant Strains (XDR) At KP

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ABSTRACT

Tuberculosis (TB) remains a main global health concern, with high rates of infection and death. Fast recognition of Mycobacterium tuberculosis (MTB) and rifampicin resistance is crucial to recover treatment results and control transmission. Out-of-date tests like AFB culture and smear microscopy have limits in speed and sensitivity. The GeneXpert MTB/RIF assay suggests rapid, accurate detection of MTB and rifampicin resistance within 90 minutes. With Pakistan facing a high load of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, this study evaluates the diagnostic accuracy of GeneXpert in pulmonary TB.1. To determine the prevalence of TB, rifampicin resistance, and associated risk factors of Mycobacterium tuberculosis among pulmonary tuberculosis (PTB). 2. Identify potential extensively drug-resistant Tuberculosis (XDR-TB) cases, informing effective treatment strategies.This study was conducted following approval from the Head of the TB Department at Timergara Teaching Hospital, Dir Lower, Khyber Pakhtunkhwa. Data were collected from patients who attended the TB Department of the timergara teaching hospital between January 2023 and December 2024. Demographic information and clinical characteristics were recorded. Sputum samples were collected in accordance with WHO guidelines and tested using the GeneXpert system to detect Mycobacterium tuberculosis complex (MTBC) and rifampicin resistance (RR). For samples that tested positive for RR, further testing for extensively drug-resistant TB (XDR-TB) was carried out to assess drug sensitivity and resistance patterns. The results were shared with physicians to guide appropriate treatment decisions. The study, conducted at Timer Garas Teaching Hospital from January 2023 to December 2024 with 7,157 participants suspected of pulmonary tuberculosis (PTB), found that 692 individuals tested positive for Mycobacterium tuberculosis complex (MTBC). The majority of participants were male (60%), and all showed PTB symptoms. A strong association was observed between certain symptoms—such as chronic cough, sputum production, fatigue, and shortness of breath—and MTBC positivity, with 100% positivity seen in those with sputum, fatigue, and breathlessness. Drug resistance testing among the 692 MTBC-positive cases revealed rifampicin resistance in 36 cases. Further extended drug resistance (XDR) analysis showed the highest resistance to Isoniazid (10 cases) and Fluoroquinolones (9), while Capreomycin and Ethionamide remained fully effective. Chi-square analysis showed statistically significant associations ($p < 0.05$) between MTBC and risk factors, including HIV (3.0%), tobacco use (5.0%), close contact with TB patients, and diabetes (6.5%). Erythrocyte sedimentation rate (ESR) levels were also elevated in MTBC-positive patients, indicating active inflammation. This study investigated 7,157 PTB-suspected patients, revealing strong relations between MTBC detection and signs like sputum production, fatigue, and shortness of breath, each showing 100%

positivity. Of the 692 MTBC-positive cases, 36 were rifampicin-resistant, with supplementary resistance to isoniazid and fluoroquinolones. Momentous links were also found between MTBC and risk facters such as HIV, diabetes, tobacco use, and close contact people with TB patients. The results highlight the need for symptom-based screening, drug resistance monitoring, and joined care for at-risk groups.

BACKGROUND

Tuberculosis remains a most important health problem, accounting for millions of new cases and deaths every year globally. Therefore, rapid detection of *Mycobacterium tuberculosis* (MTBC) and rifampicin resistance in probable TB cases is essential for early diagnosis and treatment, thereby reducing the risk of transmission of the illness, mortality rates, and rise of drug-resistant TB. AFB culture is well-thought-out, the gold standard test for closing determination of TB, but the turnaround time is 2–8 weeks, and it needs trained personnel and luxurious lab equipment[1]. Smear microscopy for acid-fast bacilli (AFB) is one of the fastest and cheapest test available, but it has poor sensitivity and poor predictive value in the diagnosis of both pulmonary and extrapulmonary tuberculosis[2, 3]. Thus, rapid identification, which is crucial for initial treatment, improves patient outcomes, and more actual public health intervention relies on nucleic acid amplification techniques. The GeneXpert MTB/RIF (rifampicin) assay is a new, joined, cartridge-based, nucleic acid amplification test (CBNAAT) for rapid diagnosis of MTBC and rapid detection of rifampicin resistance in both pulmonary and extrapulmonary samples[3, 4]. The GeneXpert (GX) test was established and launched by the Foundation for Advanced New Diagnostics (FIND) and Cepheid Corporation in 2004. Nevertheless, the development of the GeneXpert test was completed in 2008. The World Health Organization (WHO) recognized the GeneXpert for use in TB-endemic countries in December 2010, stating it was a major milestone for global judgment of tuberculosis [5]. The test isolates the *Mycobacterium* genome from captured bacteria and amplifies DNA (deoxyribonucleic acid) using polymerase chain reaction. GeneXpert test identifies a relevant 81bp (base pair) fragment of the MTBC rpoB gene using fluorescent probes called molecular beacons. GeneXpert assay senses MTBC by polymerase chain reaction (PCR) amplification of the rpo gene and determination of rifampicin resistance by subsequent probing of this region for mutations that are linked with rifampicin resistance[6, 7]. Turnaround time of the test is 90 minutes. GeneXpert needs around 130 bacilli per ml of sputum for a positive result, whereas the Ultra test needs 16 bacilli each ml of sputum for the test to be positive. It is specific for MTBC complex; i.e., it can differentiate MTBC from other mycobacteria. For each sample, the test is approved out in a locked system (cartridge), so there is a reduced risk of cross-contamination and human error. Some studies have exposed fruitful use of the GeneXpert test on pulmonary and extrapulmonary samples with high sensitivity and specificity[8, 9]. Nevertheless, fewer false positive and false negative results of GeneXpert have also been reported newly, hence we decided to study and investigate its investigative correctness in pulmonary TB.

In addition to molecular diagnostics like GeneXpert, Erythrocyte Sedimentation Rate (ESR) is one of the repetitive blood tests to determine the level of inflammation in a person's body. Inspection of the erythrocyte sedimentation rate is used to help diagnose the development of the disease and assist for the victory of long-lasting therapy, for instance, in tuberculosis. If the erythrocyte sedimentation rate is high, it indicates an active lesion, and an increase in the erythrocyte sedimentation rate associated with the previous one indicates an extensive process, while a reduced erythrocyte sedimentation rate associated with the prior one indicates a development[10].

China is one of the world's 22 countries with the highest load of tuberculosis. China is also one of the world's 27 countries with the chief load of MDR-TB/XDR-TB. The frequency of drug-resistant tuberculosis is very high [11]. According to a national survey of drug-resistant tuberculosis in China, 5.7% of innovative cases and 25.6% of formerly treated cases had MDR-TB. Roughly 8% of the patients with MDR-TB had XDR-TB [12]. Conferring to the results from a nationwide population-based study of TB in 2010 that only included those aged ≥15 years, the prevalence of TB was 459 cases per 100,000 population. The over-all rate of drug resistance was 42.1%. The frequency of MDR-TB among pulmonary TB patients in China was 6.8%. The proportion of MDR-TB cases with XDR-TB was 23.59% [13]. The study from Shandong showed that 3.6% (95%CI, 2.9%-4.6%) were multidrug-

resistant (MDR) isolates, and 20.0% (95%CI, 11.9%-31.4%) of patients with MDR-TB were XDR [14]. The prevalence of MDR- and XDR-TB is of serious concern in China.

The treatment of patients with XDR-TB is usually more complex, toxic, and costly, and less effective than the treatment of other forms of TB because of the severity of XDR-TB disease and the deficiency of effective anti-TB drugs[15].

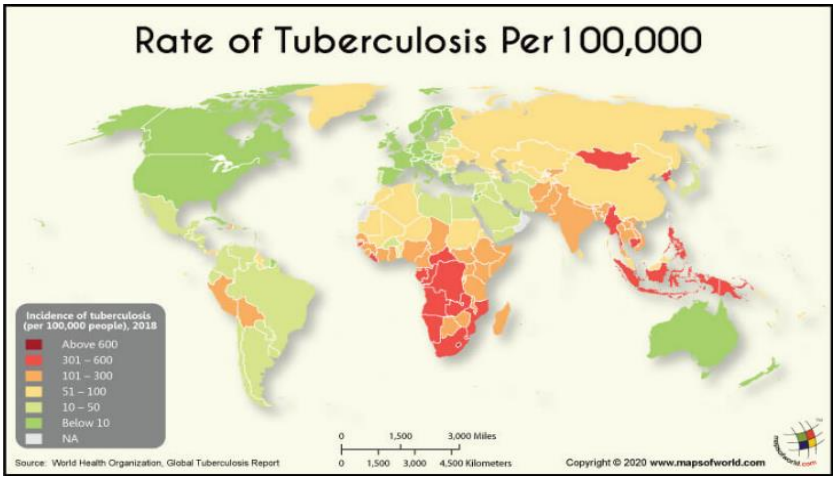

Figure 1. Global Tuberculosis Incidence (2018): Cases per 100,000 People

METHODOLOGY

A facility-based cross-sectional study was led at Timergara Teaching Hospital, Dir Lower, from January 2023 to December 2024. The source inhabitants were all PTB suspected patients attending the TB department at Timergara teaching hospital, Dir, lower. The study population included all TB-suspected patients who were enrolled in the hospital during the study period. Before the genuine specimen collection, the patients were trained about the technique for how to assemble the sputum sample as per WHO standards. The sputum sample was processed by following the SOPs of MTB/RIF Xpert assay processing techniques. First, the label of the falcon tube was checked with the patient ID number. The sample was collected, ready, and incubated by adding a reagent buffer in a 1:2 quantity sample and the reagent, correspondingly. The incubated sample was inspected to detect Mycobacterium tuberculosis by means of a GeneXpert machine, model number 110001340 (Cepheid), with the molecular technique (MTB/RIF Xpert Assay).

After the finding of Mycobacterium tuberculosis on GeneXpert, take the blood and perform HIV and ESR tests for each patient. The ESR is measured by the Westergren method, which distinguishes the sedimentation rate of red blood cells in whole blood by mixing venous blood with an EDTA and allowing the mixture to stand in an standing standard pipette (Figure 2.), interpretation the millimeters of the cells down after one hour [16]. The dependent normal range of ESR in this study is 0-22 mm/hr for men and 0-29 mm/hr for women[17] and perform the XDR test of those patients who is Rifampicin resistance detection detected according to the procedure of the test mentioned in the script placed in the box of XDR kit.

Ethical approval was gained from the ethical committee of TB department employed under the WHO at Timergara teaching hospital Dir Lower. Moreover, after explaining the status, purpose, and procedure of the study briefly, written consent was got from study participants. A parent or legal guardian providing written informed consent for any participant under the age of 18 years. Any patient who was not prepared to take part in the study had the full right not to do so, and the privacy of the study participants was also strictly maintained. Any study participant who was found to be infected with the bacterium was referred to a physician for treatment. This study was fully conducted in agreement with ethical considerations. Figure 2 illustrate the methodology of data collecation.

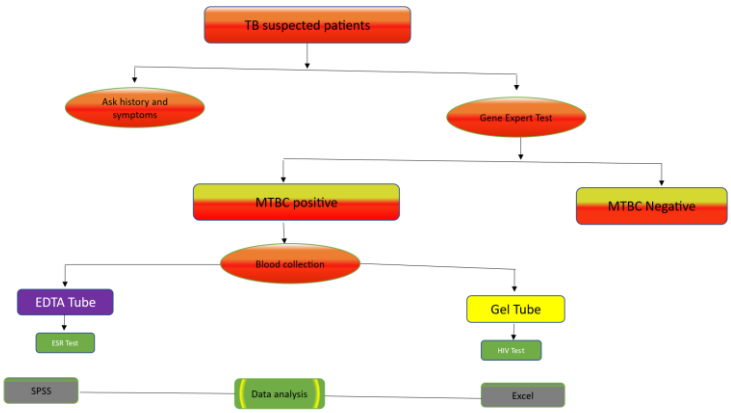

Figure 2. Methodology of data collecation

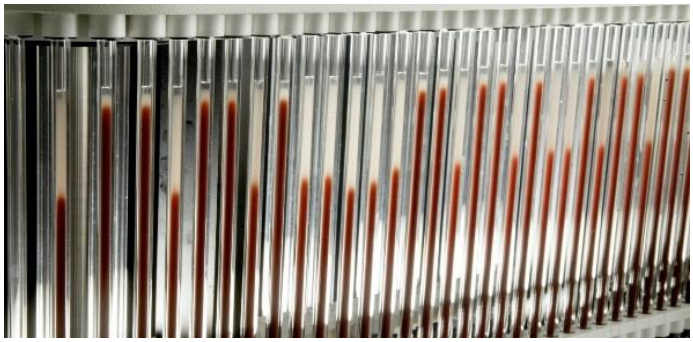

Figure 3. Westergren pipette array on Starred automated ESR analyzer

Objective

1 To evaluate the incidence of TB, rifampicin resistance, and linked risk factors of Mycobacterium tuberculosis among pulmonary tuberculosis (PTB).

2 Categorize potential extensively drug-resistant Tuberculosis (XDR-TB) cases, notifying effective treatment strategies.

RESULTS

A total of 7157 PTB-suspected people contributed in this study. Out of the entire study's contributors, approximately 60% were male and 40% female. All study participants exhibited clinical signs and symptoms of PTB. Table 1 illustrates the number and percentage of individuals who tested positive or negative for *Mycobacterium tuberculosis complex* (MTBC) based on the occurrence or absence of exact symptoms such as chronic cough, sputum production, night sweats, fatigue, shortness of breath, loss of appetite, weight loss, and fever. For each symptom, the data is divided into two groups: those who reported the symptom ("Yes") and those who did not ("No"). Inside each group, it demonstrations how many entities tested positive or negative for MTBC, laterally with the corresponding percentage. For example, among individuals with chronic cough, 94.8% tested positive for MTBC. Similarly, 100% of those with sputum production, fatigue, or shortness of breath tested positive, compared to lower percentages among those without those symptoms. The “Total No. (%)” column provides the total number of individuals with or without each symptom and the percentage of that subgroup within the total sample. This setup enables a clear comparison between symptomatic and asymptomatic individuals regarding MTBC detection.

Table 1. Clinical Signs and Symptoms of PTB Suspected Cases at Timer Gara teaching hospital, Dir from January 2023 to December 2024 (N=7157)

Parameters	**MTB Detected** (%) No	**MTB Not Detected** (%) No	**Total** (%) NO
Chronic Cough			
Yes	(94.8%) 656	(97.66%) 6314	(97.4%) 6970
No	(5.2%) 36	(2.34%) 151	(2.6%) 187

Sputum Production Yes No	(100%) 692 (0%) 0	(96.88%) 6263 (3.12%) 202	(97.2%) 6955 (2.8%) 202
Night Sweat Yes No	(97.37%) 674 (2.63%) 18	(92.99%) 6012 (7.01%) 653	(93.4%) 6686 (6.6%) 671
Fatigue Yes No	(100%) 692 (0%) 0	(97.4%) 6297 (2.6%) 168	(97.4%) 6989 (2.4%) 168
Shortness of Breath Yes No	(100%) 692 (0%) 0	(89.87%) 5810 (10.13%) 655	(90.8%) 6503 (9.2%) 655
Loss of Appetite Yes No	(92.11%) 637 (7.89%) 55	(68.83%) 4450 (31.17%) 2015	(70.9%) 5087 (29.1%) 2070
Weight Loss Yes No	(92.11%) 637 (7.89%) 55	(63.38%) 4098 (36.62%) 2367	(66%) 4735 (34%) 2422
Fever Yes No	(76.32%) 528 (23.68%) 164	(61.82%) 3999 (38.8%) 2508	(63.1%) 4527 (36.9%) 2672

Out of the 692 positive cases, rifampicin resistance was recognized 36, as illustrated in Figure 4.

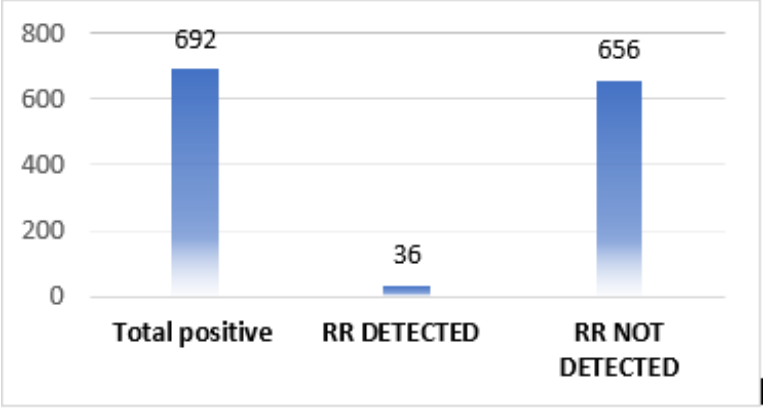

Figure 4. Distribution of RR Detection Among Positive Cases

Subsequently the RR detection XDR were performed to checked the sensitivity and resistance of different drugs that we use, especially for the treatment of TB. These drugs include Isoniazid (INH), Fluoroquinolones (FLQ), Amikacin (AMK), Kanamycin (KAN), Capreomycin (CAP), and Ethanamide (ETH). Among the drugs confirmed on Gene xpert Isoniazid and Fluoroquinolones show the highest number of resistance cases with 10 and 9, respectively, this shows that some bacterial strains are no longer successfully treated with these drugs, suggesting emerging resistance, in contrast, Amikacin and Kanamycin show no resistance, though each has 2 intermediate cases meaning that they might less effective. Capreomycin and Ethanamide perform the best and detest, with 100% sensitivity and no resistance or intermediate cases noted, indicating that they are fully effective against tested strains, as shown in Table 2.

Table 2. No of resistance, sensitive, and intermediate patients on XDR kit

Drugs Name	Resistance No	Sensitive NO	Intermediate NO
INH (Isoniazid)	10	25	1
FLQ (Fluoroquinolones)	9	24	3
AMK (Amikacin)	0	34	2
KAN (Kanamycin	0	34	2
CAP (capreomycin)	0	36	0
ETH (Ethionamide)	0	36	0

And from the MTBC detected patients, 692 who has been diagnosed with MTBC among these patients 21(3.0%) patients have HIV which means that these patients have both TB and HIV infection a chi square test is apply which show significance association between MTBC and HIV (Table 3 the p value is less than 0.05)

Table 3. Chi square test shows Significant association between MTBC and HIV

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	196.770[a]	1	<.001		
Continuity Correction[b]	186.533	1	<.001		
Likelihood Ratio	98.705	1	<.001		
Fisher's Exact Test				<.001	<.001
N of Valid Cases	7157				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.03.

b. Computed only for a 2x2 table

35(5.0%) patients are tobacco smokers, smoke can weaken the lungs and make it harder to fight off infection like TB, a chi square test is applied which show significant association between MTBC and Tobacco smokers (Table 4, the P value is less then 0.05).

Table 4. chi square test show signaficant associant between MTBC and Tobacco smokers

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	328.594[a]	1	<.001		
Continuity Correction[b]	318.283	1	<.001		
Likelihood Ratio	165.168	1	<.001		
Fisher's Exact Test				<.001	<.001
N of Valid Cases	7157				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.38.

b. Computed only for a 2x2 table

43 patients have close contact with TB patients; TB bacteria are spread through sneezes or spits release droplets that contain bacteria and a person nearby can inhale these droplets allowing the bacteria to enter their lungs can cause infection. A chi square test is applied which show significance association between MTBC and the people who have close contact with MTBC patients. Table 5 show significance association between MTBC and close contact people with MTBC patients (P value is less than 0.05).

Table 5. chi square test show significance association between MTBC and close connect people

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	404.155[a]	1	<.001		
Continuity Correction[b]	393.817	1	<.001		
Likelihood Ratio	203.389	1	<.001		
Fisher's Exact Test				<.001	<.001
N of Valid Cases	7157				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.16.

b. Computed only for a 2x2 table

And 45(6.5%) patients are diabetic and also TB, a chi square test has apply which can also show significance association between diabetes and MTBC (the P value is less then 0.05) shown in Table 6.

Table 6. chi square show significant association between MTBC and diabetes

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	423.072[a]	1	<.001		
Continuity Correction[b]	412.728	1	<.001		
Likelihood Ratio	212.972	1	<.001		
Fisher's Exact Test				<.001	<.001
N of Valid Cases	7157				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.35.

b. Computed only for a 2x2 table

The erythrocyte sedimentation rates is check from the MTBC detected patient the result has been illustrate in Figure 5.

Figure 5. Distribution of ESR Levels Among TB patients

DISCUSSION

TB is a curable disease if diagnosed early and treated effectively; thus, it is highly important to have effective and cost-effective diagnostic tests. Early diagnosis and proper treatment of TB can assist in improving cure rates, dropping transmission rates, illness, and death[18]. ZN smear microscopy conveys a high risk of false-negative results due to low sensitivity, and it doesn't help to differentiate between drug-susceptible and drug-resistant strains of MTBC**.** These are owing to poor sample quality, coupled with a need for technical expertise. Meanwhile, culture, being the gold standard test for sensing MTB, takes some weeks to yield results, and largely depends on well-equipped laboratory facilities and skilled technicians[1, 2, 19]. Nucleic-acid-amplification tests provide more appropriate and accurate diagnosis of TB, thereby contributing to early beginning of TB treatment. GeneXpert test is an joined, fully automated nucleic-acid-amplification test that detects MTBC and rifampicin resistance within 90 minutes. GeneXpert is a gifted test with high sensitivity, specificity, and rapid reversal time [3, 4, 20]. The test is highly effective in pulmonary TB cases when compared to extrapulmonary cases. In addition, the GeneXpert test is likely to advance the indicative accuracy of TB at homes where AFB culture or DST facilities are not exist.

The result showed that the overall prevalence of PTB among suspected cases was 9.6%, which agrees with the studies conducted in Ethiopia [21, 22]. In comparison with the present study, a higher rate of prevalence of TB was reported in a study conducted in 2013 in Pakistan at dir , which was very high 44%[23]. Another study done which shows 38.51%[24]. In 2023, most individuals who developed TB were in the WHO regions of Southeast Asia (45%), Africa (24%), and the Western Pacific (17%), with smaller proportions in the Eastern Mediterranean (8.6%), [25], Nigeria (22.9%)[26], in Addis Ababa (15.11%)[27], Gambella regional state, southwest Ethiopia (20%)[28].

The difference may be due to variations in the nature of the study populations, study settings, and study designs. In contrast, other studies had lower reports of the prevalence, 2.6%[29], 4.6%[30], 6.5%[31]. This lower prevalence, in contrast with the current study, might be due to variation in the study setting and study population.

In the current study, the rate of TB/HIV co-infection was 21/692. According to WHO, which indicates that 8% of new HIV cases are diagnosed[32]. Some studies diagnosed high. In contrast, a higher TB/HIV co-infection result was reported from several studies, like in a systematic review report (22%) [33]. A still higher rate of TB/HIV co-infection was reported from MDR-TB suspected cases (56.6%)[34], in our study 45 (6.5%) are diabetic out of 692 Tb patients and the other study publish in 2019 which 12.5% diabetic patients suffer from TB[35]. the 35 patients are tobacco smokers which can weak their lungs and make it harder for him to fight infeecation like Tb, the data is very accoding to region to region for example the study done it pakitan it 2020 which is 22.5%[36]. And those patients who is close contect to the Tb patients is 35 is suffer from MTBC in our study. After that chi squre test is apply on these risk facter to check the association between the diabetes, HIV, Tobacco smokers and close contect people with MTBC, which can show that there is association between the MTBC and these risk facters, the other studies done which also show the asociation between MTBC and these risk facters [37, 38].

Rifampicin-resistant PTB isolates found by GeneXpert were strong analysts o f MDR-TB[31]. Even though the number of RR-TB (36/692; 5.2%). in the present study were less than in similar studies in Ethiopia[27, 39], and in Nigeria[26]. This can still imply a serious public health problem in the study area. The study was done in the Ethiopian region of Gambella[28], which is comparable to our current study. This indicates a limited circulation of the MDR-TB into the community.

the ESR is a blood test measuring the rate of reduction of red blood cells in a column of anticoagulated blood in 1 hour, with the units stated in milliliters per hour (mm/h).[40]. In experimental practice, it is regularly done as a generic test for an extensive range of pathological circumstances such as acute or chronic infections, systemic inflammatory situations, and neoplastic conditions. The ESR is typically raised in such conditions, and infections, collagen diseases, metastatic malignant tumours, and renal disease are said to be the leading causes of higher values ≥ 100 mm/h[40, 41]. According to our study ESR value is high then 100 in 50%TB patients, in between 51 to 100 is 25% patients and 1 to 50 is also 25% value of ESR in TB patients and A South African study done on the causes of high ESR in an in patient population examined 419 ESR results with values ≥ 100mm/h (Westergren) in a medical ward at Kalafong Hospitalin Pretoria. The study found that the most common disease condition associated with such high ESR values was infection, and that the most common infection was TB. A study undertaken in Qatar to evaluate the utility of ESR in the diagnosis of childhood TB studied 144 childhood TB cases retrospectively and found that 68 (47%) of the 144 cases had ESR results documented at the time of the TB diagnosis, that 22 (33%) of the 68 cases had a normal ESR (< 10 mm/h) and 46(67%) had elevated values (> 10 mm/h), and that symptomatic and culture-positive TB cases had significantly higher ESR values than asymptomatic and culture-negative cases[42]. Another study, conducted in India, investigated the ESR as a possible predictive indicator for screening TB patients for HIV infection[43]..

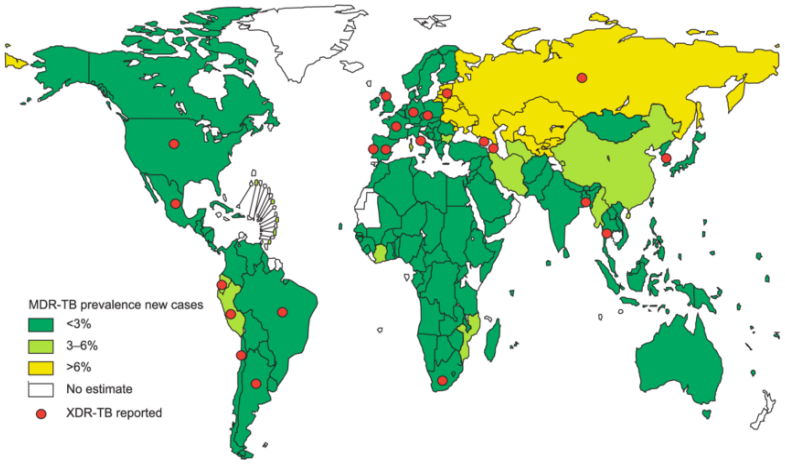

Figure 6. prevalence of MDR cases in 2024 according to WHO

CONCLUSIONS

This study presented, at Timergara Teaching Hospital from January 2023 to December 2024, studied 7,157 PTB-suspected people, of whom 60% were male and 40% female, all showing clinical signs of tuberculosis. A strong association was found between MTBC detection and specific symptoms, with sputum production, fatigue, and shortness of breath each showing a 100% positivity rate, while chronic cough, night sweats, and loss of appetite also showed high detection rates. Out of 692 MTB-positive cases, 36 were rifampicin-resistant, and additional testing revealed resistance primarily to isoniazid (10 cases) and fluoroquinolones (9 cases), while capreomycin and ethionamide remained fully effective with no resistance or intermediate cases. Significant associations ($p < 0.05$) were observed between MTBC and risk factors such as HIV (3.0%), diabetes (6.5%), tobacco use (5.0%), and close contact people with TB patients, representing these as key risk factors. Elevated ESR levels were also noted among positive cases, reflecting active inflammation. Overall, the findings highlight the importance of symptom-based screening, drug resistance surveillance, and combined management of comorbidities to enhance TB diagnosis and control efforts.

REFRENCES

1.Luetkemeyer, A.F., et al., *Evaluation of Xpert MTB/RIF versus AFB smear and culture to identify pulmonary tuberculosis in patients with suspected tuberculosis from low and higher prevalence settings.* Clinical Infectious Diseases, 2016. 62(9): p. 1081-1088.

2.Walusimbi, S., et al., *Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis.* BMC infectious diseases, 2013. 13(1): p. 507.

3.Lawn, S.D. and M.P. Nicol, *Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance.* Future microbiology, 2011. 6(9): p. 1067-1082.

4.Marlowe, E.M., et al., *Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis complex in respiratory specimens.* Journal of clinical microbiology, 2011. 49(4): p. 1621-1623.

5.WHO, *Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational "How-to"; practical considerations.* WHO, StopTB, 2011.

6.Hillemann, D., et al., *Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system.* Journal of clinical microbiology, 2011. 49(4): p. 1202-1205.

7.Blakemore, R., et al., *Evaluation of the analytical performance of the Xpert MTB/RIF assay.* Journal of clinical microbiology, 2010. 48(7): p. 2495-2501.

8.Maynard-Smith, L., et al., *Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review.* BMC infectious diseases, 2014. 14(1): p. 709.

9.Sharma, S.K., et al., *Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis.* PloS one, 2015. 10(10): p. e0141011.

10.Sofyanita, E.N., *Correlation Of Erythrocyte Sedimentation Rate (ESR) and Lymphocyte Counts in Pulmonary Tuberculosis Patients during Intensive Treatment in Purwokerto City: Hubungan Nilai Laju Endap Darah (LED) dengan Jumlah Limfosit pada Pasien Tuberkulosis Paru Masa Pengobatan Tahap Intensif di Kota Purwokerto.* Borneo Journal of Medical Laboratory Technology, 2021. 4(1): p. 249-254.

11.Organization, W.H., *Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response*, in *Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response*. 2010. p. 71-71.

12.Zhao, Y., et al., *National survey of drug-resistant tuberculosis in China.* New England Journal of Medicine, 2012. 366(23): p. 2161-2170.

13.Tuberculosis, N.T.S.G.o.t.E.S.S.f., *Implementing Office of the Epidemiological Sampling Survey for Tuberculosis (2012) The prevalence of pulmonary tuberculosis in a national survey across China in 2010.* Zhonghua Jie He He Hu Xi Za Zhi. 35(9): p. 665-658.

14.Li, X., et al., *Population-based surveillance of extensively drug- resistant tuberculosis in Shandong Province, China.* The International journal of tuberculosis and lung disease, 2012. 16(5): p. 612-614.

15.Jacobson, K.R., et al., *Treatment outcomes among patients with extensively drug-resistant tuberculosis: systematic review and meta-analysis.* Clinical infectious diseases, 2010. 51(1): p. 6-14.
16.Heritage, A., *The American heritage medical dictionary*. 2008: Houghton Mifflin Harcourt.
17.Raheem, A.H., *Erythrocyte sedimentation rate in patients with positive sputum for AFB and negative HIV serological test.* Muthanna Medical Journal, 2025. 3(1): p. 17-23.
18.Yayan, J., et al., *Early detection of tuberculosis: a systematic review.* Pneumonia, 2024. 16(1): p. 11.
19.Opota, O., et al., *Added value of molecular assay Xpert MTB/RIF compared to sputum smear microscopy to assess the risk of tuberculosis transmission in a low-prevalence country.* Clinical microbiology and infection, 2016. 22(7): p. 613-619.
20.Chang, K., et al., *Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis.* Journal of Infection, 2012. 64(6): p. 580-588.
21.Nugussie, D.A., G.A. Mohammed, and A.T. Tefera, *Prevalence of Smear-Positive Tuberculosis among Patients Who Visited Saint Paul's Specialized Hospital in Addis Ababa, Ethiopia.* BioMed research international, 2017. 2017(1): p. 6325484.
22.Tulu, B., et al., *Smear positive pulmonary tuberculosis and its risk factors among tuberculosis suspect in South East Ethiopia; a hospital based cross-sectional study.* BMC research notes, 2014. 7(1): p. 285.
23.Ahmad, T., et al., *Prevalence of tuberculosis infection in general population of district Dir (lower) Pakistan.* Middle East J Sci Res, 2015. 23(1): p. 14-17.
24.Ahmad, T., *Epidemiology of tuberculosis: current status in district Dir (lower) Pakistan.* Int J Sci Eng Res, 2013. 4(11): p. 755-63.
25.Chen, Z., et al., *Decoding the WHO global tuberculosis report 2024: a critical analysis of global and Chinese key data.* Zoonoses, 2025. 5(1): p. 999.
26.Ikuabe, P.O. and I.D. Ebuenyi, *Prevalence of rifampicin resistance by automated Genexpert rifampicin assay in patients with pulmonary tuberculosis in Yenagoa, Nigeria.* The Pan African Medical Journal, 2018. 29: p. 204.
27.Arega, B., F. Menbere, and Y. Getachew, *Prevalence of rifampicin resistant Mycobacterium tuberculosis among presumptive tuberculosis patients in selected governmental hospitals in Addis Ababa, Ethiopia.* BMC infectious diseases, 2019. 19(1): p. 307.
28.Ejeta, E., et al., *Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and Rifampicin resistance in high human immunodeficiency virus setting in gambella regional state, southwest ethiopia.* Journal of clinical tuberculosis and other Mycobacterial diseases, 2018. 12: p. 14-20.
29.Semunigus, T., et al., *Smear positive pulmonary tuberculosis and associated factors among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia.* Annals of clinical microbiology and antimicrobials, 2016. 15(1): p. 50.
30.Eliso, E., G. Medhin, and M. Belay, *Prevalence of smear positive pulmonary tuberculosis among outpatients presenting with cough of any duration in Shashogo Woreda, Southern Ethiopia.* BMC Public Health, 2015. 15(1): p. 112.
31.Sinshaw, W., et al., *Prevalence of tuberculosis, multidrug resistant tuberculosis and associated risk factors among smear negative presumptive pulmonary tuberculosis patients in Addis Ababa, Ethiopia.* BMC infectious diseases, 2019. 19(1): p. 641.
32.Organization, W.H., *World Health Organization Global Tuberculosis Report 2020.* World Health Organization, 2020. 232.
33.Teweldemedhin, M., et al., *Tuberculosis-Human Immunodeficiency Virus (HIV) co-infection in Ethiopia: a systematic review and meta-analysis.* BMC infectious diseases, 2018. 18(1): p. 676.
34.Mesfin, E.A., et al., *Drug-resistance patterns of Mycobacterium tuberculosis strains and associated risk factors among multi drug-resistant tuberculosis suspected patients from Ethiopia.* PloS one, 2018. 13(6): p. e0197737.
35.Restrepo, B.I., *Diabetes and tuberculosis.* Understanding the host immune response against mycobacterium tuberculosis infection, 2018: p. 1-21.
36.Marshall, A.-M., et al., *Smoking prevalence among tuberculosis patients: A crosssectional study in Bangladesh and Pakistan.* Tobacco induced diseases, 2020. 18: p. 70.
37.Narasimhan, P., et al., *Risk factors for tuberculosis.* Pulmonary medicine, 2013. 2013(1): p. 828939.
38.Etim, N.G., et al., *Risk factors of tuberculosis and strategies for prevention and control.* Int J Innov Healthcare Res, 2024. 12(1): p. 1-3.

39.Jaleta, K.N., et al., *Rifampicin-resistant Mycobacterium tuberculosis among tuberculosis-presumptive cases at University of Gondar Hospital, northwest Ethiopia.* Infection and drug resistance, 2017: p. 185-192.
40.Brigden, M.L., *Clinical utility of the erythrocyte sedimentation rate.* American family physician, 1999. 60(5): p. 1443-1450.
41.Levay, P.F. and M. Viljoen, *Erythrocyte sedimentation rates equal to or above 100 mm/h in the pre-AIDS era.* 2002.
42.Al-Marri, M. and M. Kirkpatrick, *Erythrocyte sedimentation rate in childhood tuberculosis: is it still worthwhile?* The International Journal of Tuberculosis and Lung disease, 2000. 4(3): p. 237-239.
43.Sarkar, K., et al., *Erythrocyte sedimentation rate may be an indicator for screening of tuberculosis patients for underlying HIV infection, particularly in resource-poor settings: an experience from India.* 2013.