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DETECTION OF MYCOBACTERIUM TUBERCULOSIS AND RIFAMPICIN RESISTANCE BY GENE XPERT METHOD AMONG EXTRAPULMONARY CLINICAL SPECIMENS-A CROSS SECTIONAL STUDY

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ABSTRACT

Background: Extrapulmonary tuberculosis (EPTB) poses significant diagnostic challenges due to its nonspecific clinical presentation and paucibacillary nature of specimens. Rapid detection of Mycobacterium tuberculosis (MTB) and associated drug resistance is crucial for early treatment initiation and control of transmission. The Gene Xpert MTB/RIF assay is a rapid molecular diagnostic tool for detecting MTB and rifampicin resistance.

Aim: To assess the diagnostic utility of the Gene Xpert MTB/RIF assay for the detection of Mycobacterium tuberculosis and rifampicin resistance in extrapulmonary clinical specimens.

Materials and Methods: This cross-sectional study was conducted over one year in the TB CDST Laboratory, Department of Microbiology, Gajra Raja Medical College, Gwalior. A total of 100 extrapulmonary specimens, including pus, pleural fluid, cerebrospinal fluid (CSF), ascitic fluid, FNAC, tissue biopsy, bone marrow, and pericardial fluid, were analyzed using the Gene Xpert MTB/RIF assay. Demographic details were recorded, and statistical analysis was performed using the Chi-square test, with $p < 0.05$ considered significant.

Results: The overall MTB positivity rate was 31%. Among MTB-positive cases, 83.87% were rifampicin-susceptible, while 16.13% showed rifampicin resistance. CSF samples demonstrated the highest MTB positivity rate (44.44%), followed by pus (37.5%) and pleural fluid (20%). The assay showed an overall sensitivity of 89.66%, specificity of 92.96%, and accuracy of 92%. MTB detection was significantly higher in females and in the 11–20-year age group ($p < 0.05$). Rifampicin resistance showed no statistically significant association with age or gender.

Conclusion: The Gene Xpert MTB/RIF assay is a rapid, sensitive, and reliable diagnostic tool for extrapulmonary tuberculosis, with the added advantage of early detection of rifampicin resistance. Its routine use in extrapulmonary specimens can facilitate timely diagnosis, appropriate therapy, and improved TB control, particularly in high-burden settings.

Keywords: *Mycobacterium tuberculosis, Gene Xpert, MTB/RIF assay, extrapulmonary samples.*

INTRODUCTION

Tuberculosis (TB) remains a significant global health challenge, with an estimated incidence rate of 8.8 million new cases annually and a mortality rate of approximately 1.3 million deaths per year [1]. Tuberculosis affecting organs other than the lungs, such as lymph nodes, pleura, bones and joints, genitourinary system, central nervous system, abdomen, and skin, is referred to as extrapulmonary tuberculosis. In immunocompromised people, particularly those with HIV infection, the frequency of EPTB can rise to 40–50% from 15–20% in immunocompetent individuals [2]. EPTB frequently resembles other inflammatory or malignant illnesses, has a nonspecific clinical appearance, and varies depending on the region of involvement [3]. Because many extra-pulmonary TB specimens are paucibacillary and frequently smear negative, which reduces the sensitivity of traditional diagnostic techniques, laboratory diagnosis presents an additional difficulty [4, 5]. A real-time PCR-based test called the GeneXpert assay makes it possible to quickly identify *Mycobacterium tuberculosis*, even in extra-pulmonary specimens. Early detection of active extra-pulmonary tuberculosis enables prompt treatment initiation, potentially lowering the disease burden. Nucleic acid amplification techniques (NAATs), on the other hand, provide quick and sensitive detection of *Mycobacterium tuberculosis*, which is essential for the early diagnosis and treatment of TB, especially in high-risk populations like immunocompromised people or those who have previously come into contact with active TB cases. These methods aid in lowering transmission rates in addition to facilitating prompt treatment [6, 7]. The GeneXpert MTB/RIF assay, an automated real-time polymerase chain reaction system that improved TB control operations primarily in resource-constrained locations, was authorized by the World Health Organization. In less than two hours, our assay simultaneously detects *Mycobacterium tuberculosis* (MTB) and identifies rifampicin resistance (RR) [8]. The Xpert MTB/RIF assay has been widely used in TB diagnosis algorithms due to numerous studies that have shown its high sensitivity and specificity in pulmonary specimens [9]. The Xpert MTB/RIF assay's usefulness in extrapulmonary specimens, such as lymph node aspirates, pleural fluid, cerebrospinal fluid, pus, tissue biopsies, and other bodily fluids, has been assessed more frequently in recent years. For several types of extrapulmonary tuberculosis, especially tuberculous lymphadenitis and TB meningitis, when prompt detection is crucial, the World Health Organization has approved the use of Xpert MTB/RIF as an initial diagnostic test [10]. It is necessary to evaluate the usefulness of Xpert MTB/RIF in routine diagnosis of extrapulmonary tuberculosis (EPTB) given the difficulties in diagnosing this condition and the increasing prevalence of rifampicin-resistant TB [11].

Aim: The purpose of this cross-sectional study is to assess the diagnostic usefulness of the GeneXpert technique in a tertiary care context and to identify *Mycobacterium tuberculosis* and rifampicin resistance in extrapulmonary clinical specimens.

MATERIAL AND METHODS

This cross observational study was carried out in TB CDST Laboratory, Department of Microbiology at Gajra Raja Medical College, Gwalior, Madhya Pradesh. Study duration was one year from November 2023 to October 2024.
A total of 100 extrapulmonary samples such as pus, ascitic fluid, cerebrospinal fluid (CSF), lymph nodes, tissue biopsies, bone marrow, pericardial fluid, and pleural fluid received in our laboratory during the study period were analysed.

Inclusion criteria:

- Patients of all age group with both gender
- Extrapulmonary samples received in our laboratory for Gene Xpert MTB/RIF assay
- Patients who provided written informed consent for the study

Exclusion criteria:

- Pulmonary samples from suspected TB patients
- Individuals who did not give the consent to participate in this study

Specimen collection and processing: After receiving informed consent, all samples from patients suspected of having extrapulmonary tuberculosis were gathered in sterile, covered, leak-proof containers. All clinical specimens were processed in accordance with standard biosafety protocol due to the risk of aerosol generation. Safe laboratory practices were strictly followed to prevent contamination

Patient socio-demographic details were noted then each sample processed for Gene Xpert.

Gene Xpert MTB/RIF Assay: After adding 4 mL of sample reagent in a 2:1 ratio to 2 ml of the sample in a 50 ml Falcon tube, the tube was incubated at room temperature for 15 minutes while being periodically vortex. To ensure there were no air bubbles, the cartridge was filled with two milliliters of the mixture. Following sealing, the cartridge was put into the module, the patient ID was input, and the barcode was scanned.

Interpretation of results: The Gene Xpert System automatically analyzes fluorescent signals using preset algorithms; assay settings are fixed and cannot be altered by users. The assay concurrently identifies MTB complex and rifampicin resistance by amplifying a specific region of the rpo B gene. It uses five distinct molecular beacons (Probes A–E), each labeled with a unique fluorophore, to detect mutations within the rifampicin resistance-determining region (RRDR).

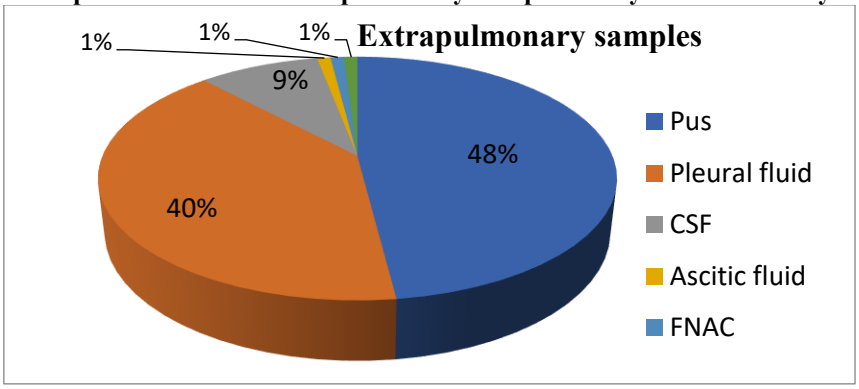
Ethical consideration: This study was approved by the Ethical Committee of the Institute.

Statistical analysis: Statistical analysis was carried out using JAMOVI version 2.6.13. The Chi-square test was applied to determine the significance of relationships among categorical variables. A p value <0.05 considered as statistically significant

RESULTS:
In our study, 100 EPTB samples were received and analysed by Gene Xpert method for detecting MTB and rifampicin resistance.

Among total samples, pus was the most commonly collected specimen (48%), followed by pleural fluid (40%) and CSF (9%).

Graph 1: Varieties of Extrapulmonary Samples Analyzed in this Study



The overall MTB positivity rate was 31%, the majority (26 cases, 83.87%) were found to be rifampicin-susceptible, while 5 cases (16.13%) exhibited rifampicin resistance. CSF exhibited the highest MTB positivity rate (44.44%) and 25% demonstrated rifampicin resistance followed by Pus samples accounted for 37.5%, MTB detection and 16.6% cases were resistant to rifampicin

Table 1: Detection of MTB & Rifampicin Resistance in Extrapulmonary Samples

Type of Sample (n*)	MTB detected (%)	RIF Resistance detected (%)	RIF Resistance not detected (%)
Pus (48)	18 (37.5%)	03 (16.67%)	15 (83.33%)
Pleural fluid (40)	08 (20%)	01 (12.50%)	07 (87.50%)
CSF (09)	04 (44.44%)	01 (25%)	03 (75%)
Ascitic fluid (01)	01 (100%)	0 (0%)	01 (100%)
FNAC (01)	0 (0%)	NA*	NA*
Tissue biopsy (01)	0 (0%)	NA*	NA*
Total (100)	**31 (31%)**	**05 (16.13%)**	**26 (83.87%)**

***n= no. of samples, NA*=Not applicable**
Gene Xpert showed a sensitivity of 89.66%, specificity of 92.96%, and overall accuracy of 92%.

Table 2: Sensitivity and specificity of Gene Xpert for MTB Detection

Type of Sample	Sensitivity%	Specificity%
Pus	100%	90.91%
Pleural fluid	85.71%	93.94%
CSF	80%	100%
Ascitic fluid	100%	Undefined
FNAC	Undefined	100%
Tissue biopsy	0%	Undefined

The highest detection rates were in the 11–20 years group, detecting MTB in 68.75% and rifampicin resistance in 27.27% cases. Detection rates declined with age, with no cases detected in children (0–10 years) or older adults (61–80 years). Statistical analysis showed significant differences in MTB detection but no significant variation in rifampicin resistance across age groups (table 3).

Table 3: Detection of MTB and RIF resistance in different age groups

Age in years	No of samples	MTB Detected	RIF resistance detected
		No. (%)	No. (%)
0-10	08	0	0
11-20	16	11 (68.75%)	03 (27.27%)
21-30	26	11 (42.31%)	01 (9.09%)
31-40	21	05 (23.81%)	01 (20%)
41-50	09	02 (22.22%)	0
51-60	12	02 (16.67%)	0
61-80	08	0	0
p value		**0.002**	**0.692**

MTB detection rates were higher among females (51.43%) as compared to males (20%), while rifampicin resistance was higher in males (23.08%) compared to females (11.11%). Significant difference in MTB detection ($p<0.05$) but no significant difference in rifampicin resistance ($p>0.05$)

Table 4: Detection of MTB and RIF resistance among gender

Gender	No of samples	MTB Detected	RIF resistance detected
		No. (%)	No. (%)
Male	65	13 (20%)	3 (23.08%)
Female	35	18 (51.43%)	2 (11.11%)
P value		**0.001**	**0.371**

DISCUSSION

TB is still a serious worldwide health concern. By identifying MTB and rifampicin resistance in less than two hours, the Gene Xpert MTB/RIF assay-a quick molecular test that was first created for pulmonary TB has now shown promise in the diagnosis of EPTB.

In our study, pus was the most common extrapulmonary specimen, followed by pleural fluid. This reflects the clinical predominance of tubercular lymphadenitis and pleural TB at our center. Our findings partially align with Kumar B. et al [12] and Chander et al [13] also reported a predominance of pus and pleural fluid among EPTB samples. Overall, these comparisons underscore that pus and pleural fluid remain key specimens in diagnosing extrapulmonary TB, despite regional and clinical variations.

We have found that the MTB detection was higher in females and Rifampicin resistance was higher in males, in agreement with the Sanjay et al [14]. The higher detection in females may reflect delayed presentation or more advanced disease whereas greater drug resistance in males was possibly due to treatment delays or non-adherence. This study highlights the need for gender-responsive TB strategies to ensure timely diagnosis and effective care for all.

Age-wise analysis revealed the highest MTB detection rate in the 11–20-year age group, with a statistically significant association ($p< 0.05$). Detection rates declined with increasing age, and no MTB was detected in children below10 years or adults above 60 years. These findings consistent with the Bankar S, et al [15] and Azadi F, et al [16], may reflect differences in immune response, exposure patterns, and healthcare-seeking behaviour across age groups. Rifampicin resistance did not show a statistically significant association with age ($p>0.05$), likely due to the small number of resistant cases.

The overall MTB positivity rate in our study was 31% in extrapulmonary specimen, among that CSF showed the highest MTB positivity rate, followed by pus and pleural fluid. These results are comparable with the Hillemann, et al [17] and Desai M, et al [18]. The higher positivity in CSF may be attributed to the targeted testing of clinically suspected tuberculous meningitis cases, where rapid molecular detection is particularly crucial due to high morbidity and mortality. Rifampicin resistance was detected in 16.13% of Gene Xpert positive cases. Pus samples accounted for the majority of rifampicin-resistant cases, which may reflect chronic disease, previous incomplete treatment, or delayed healthcare access. Comparable rifampicin resistance rates in extrapulmonary TB have been documented in earlier Indian studies, like Jagadevi, et al [19] and Murhar, R, et al [20], reinforcing the growing concern of drug-resistant TB beyond pulmonary involvement.

The performance of the Xpert MTB/RIF assay in our study, with a sensitivity of 89.66% and specificity of 92.96%, aligns well with findings from several previous studies conducted across diverse settings. For instance, comparable sensitivity and specificity were reported by Iram S, et al [21] and Terzi HA et al [22]. Overall, our results support the robust diagnostic utility of Xpert MTB/RIF in extrapulmonary tuberculosis, consistent with a broad body of international evidence.

CONCLUSION:

This study emphasizes the crucial role of the Gene Xpert MTB/RIF assay in the rapid and reliable detection of extrapulmonary tuberculosis. Gene Xpert showed high sensitivity and specificity and effectively identifies *Mycobacterium tuberculosis* and rifampicin resistance. Gene Xpert markedly shortens diagnostic time, essential in EPTB where early detection is challenging. Its concurrent identification of rifampicin resistance enables timely treatment and aids in controlling the spread of drug-resistant TB.This study supports the routine use of Gene Xpert for extrapulmonary TB, particularly in high-burden settings.

REFERENCES:

1. Global Tuberculosis Report 2013. Geneva, World Health Organization, 2017.
2. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res. 2004; 120(4):316–353.
3. Golden MP, Vikram HR. Extrapulmonary tuberculosis: An overview. Am Fam Physician. 2005; 72(9):1761–1768.
4. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010; 363: 1005-1015.
5. Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert(R) MTB/RIF assay. J. Clin. Microbiol. 2010; 48: 321348.
6. Habous M, Elimam MA, Kumar R, Deesi ZA. Evaluation of GeneXpert *Mycobacterium tuberculosis*/Rifampin for the detection of *Mycobacterium tuberculosis* complex and rifampicin resistance in nonrespiratory clinical specimens. International Journal of Mycobacteriology. 2019; 8(2): 132.
7. Kohli M, Schiller I, Dendukuri N, Dheda K, Denkinger CM, Schumacher SG, Steingart KR. Xpert MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance. Cochrane Database Syst Rev. 2018 Aug 27;8(8):CD012768. doi: 10.1002/14651858.CD012768.pub2. Update in: Cochrane Database Syst Rev. 2021 Jan 15; 1:CD012768. doi: 10.1002/14651858.CD012768.
8. Van Rie A, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert® MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? Expert Rev Mol Diagn. 2010;10(7):937–46. doi:10.15 86/erm.10.66
9. Steingart KR, Sohn H, Schiller I, et al. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2013; 1:CD009593.
10. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance (Xpert MTB/RIF): Policy update. Geneva: WHO; 2013.
11. Hamed, Amal H.G. Ahmed, Mona M. Al Adawy, Eman R.A. Fathy, Rehab M.M. Evaluation of the Gene Xpert *Mycobacterium tuberculosis*/resistance to rifampicin assay in rapid diagnosis of tuberculosis and identification of rifampin resistance in tuberculous patients. The Egyptian Journal of Chest Diseases and Tuberculosis 74(1): p 113-120, January-March 2025. | DOI: 10.4103/ecdt.ecdt_55_24
12. Kumar B, Muni S, Kumar R, Prabhat AK, Kumar S, & Kumari N. Comparative study of different diagnostic methods for detection of mycobacteria in clinically suspected patients of extrapulmonary tuberculosis at IGIMS, Patna. International Journal of Health Sciences. 2022; 6(S1):887-897.
13. Chander V, Raina SK, Bhardwaj AK, Gupta A, Kashyap S, & Sood A. Clinico epidemiological profile of extrapulmonary tuberculosis: A report from a high prevalence state of northern India. International Journal of Tropical Medicine. 2012; 7(3):117-120
14. Sanjay MG, Radha PM, Babu NP, Sushant M, & Jitesh A Genotypic diagnosis of extrapulmonary tuberculosis-CBNAAT a novel tool. Med Pulse Int J Med. 2017;4(2):79-82
15. Sheetal Bankar, Reena Set, Disha Sharma, Daksha Shah, Jayanthi Shastri, Diagnostic Accuracy of Xpert MTB/RIF Assay in Extrapulmonary Tuberculosis, Indian Journal of Medical Microbiology, Volume 36, Issue 3,2018,Pages 357-363,ISSN 0255-0857.
16. Azadi F, Rezanezhadi M, Bagheri H, Alhusseini L B, Joshaghani H R. Identification of *Mycobacterium tuberculosis* and Rifampin Resistance in Pulmonary and Extra-pulmonary Clinical Specimens Using the Gene Xpert MTB/RIF Assay. mljgoums 2021; 15 (4) :1-5
17. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbiol. 2011 Apr; 49(4):1202-5. doi: 10.1128/JCM.02268-10. Epub 2011 Jan 26. PMID: 21270230; PMCID: PMC3122824

18. Desai M, Patel M, Leuva AT. Role of automated GenexpertMTb/Rif system for rapid detection of *Mycobacterium tuberculosis* and Rifampicin resistance in extrapulmonary tuberculosis: A prospective study [Internet]. *IP Indian J Immunol Respir Med*. 2020 [cited 2025 Dec 15]; 5(1):47-51.
19. Dr. Jagadevi, Dr. Sumanta A, Dr. Shubha D S., Dr. Sudhindra K.S., & Dr. Basavaraj Sangolli. (2018). Evaluation of GeneXpert MTB/RIF assay in diagnosis of extrapulmonary tuberculosis and rifampicin resistance. *Tropical Journal of Pathology and Microbiology*, *4*(7), 478–485. https://doi.org/10.17511/jopm.2018.i07.01
20. Murhar, R., Gajbhiye, S., Zodpey, S. S., & Lichade, S. (2023). Evaluation of *Mycobacterium tuberculosis* by conventional methods, GeneXpert and line probe assay in cases of extrapulmonary tuberculosis. *International Journal of Research in Medical Sciences*, *11*(6), 2082–2089. https://doi.org/10.18203/2320-6012.ijrms 2023 1622
21. Iram S, Zeenat A, Hussain S, Yusuf NW, Aslam M. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay-Report from a developing country. Pakisthan Journal of Medical Science 2015; 31(1): 105- 11
22. Terzi HA, Aydemir Ö, Karakeçe E, Demiray T, Köroğlu M, Altındiş M. Evaluation of the Xpert MTB/RIF Test Performance in the Diagnosis of Suspected M. tuberculosis in Pulmonary and Extrapulmonary Clinical Specimens. Mediterr J Infect MicrobAntimicrob. 2024; 13:23055.13.