DETECTION OF RIFAMPICIN RESISTANT MYCOBACTERIUM TUBERCULOSIS ISOLATES USING GENEXPERT IN CLINICAL SPECIMENS OF VARIOUS BODY FLUIDS RECEIVED IN CMH, MULTAN

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Abstract

There is a higher burden of tuberculosis in low- and middle-income countries. Improved and rapid diagnostic testing, using Xpert MTB/RIF that detects both tuberculosis and rifampicin resistance. This frequency study was conducted at Microbiology Department of Combined Military Hospital, Multan where MTB GeneXpert assay was performed as per recommended guidelines. Frequency of tuberculosis was found to be 18.3% and frequency of Rifampicin resistance as 9.3 %. Tuberculosis endemic countries like Pakistan where MDR and XDR tuberculosis is prevalent at increased frequency, there is increased need for early, rapid and accurate diagnostics tools for tuberculosis diagnosis, treatment and follow up to end this endemic.

INTRODUCTION

Tuberculosis is one of the world's deadliest communicable diseases that is caused by Mycobacterium *tuberculosis* (Brown et al., 2021). There is higher burden of tuberculosis in low- and middle-income countries. Improved and rapid diagnostic testing, using Xpert MTB/RIF that detects both tuberculosis and rifampicin resistance, is limited by resource-related implementation challenges in such countries (Agarwal et al.,2016).

Pakistan ranks 5th among high burden countries where approximately 510000 people of all ages receive infection with M.tuberculosis killing almost 70000 children per year.(Rehman et al., 2020).

Early diagnosis and treatment is important tool to prevent its spread. Diagnosis of TB can be done by radiological and laboratory investigations such as finding of consolidation in the lung apices through X-Ray, Tuberculin skin test (TST) and immune-chromatographic techniques. In developing countries like Pakistan, acid fast bacilli (AFB) detection on microscopy is routinely used which is an inexpensive and rapid test for TB diagnosis however it has limitation of low sensitivity and specificity on differentiating M. *tuberculosis* from the other mycobacterium (khan et al .,2016).

GeneXpert is a molecular assay which diagnoses pulmonary tuberculosis and simultaneously detects

Rifampicin resistance. As compared to conventional methods, GeneXpert is a sensitive and rapid method for early detection of tuberculosis (Shah et al .,2019).
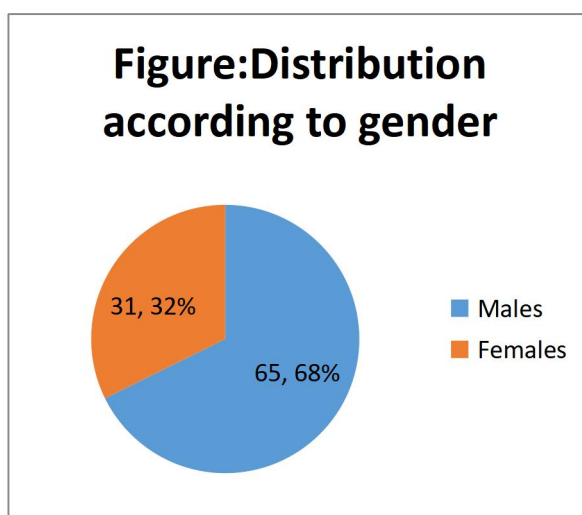
MATERIAL AND METHODOLOGY:

This frequency study was conducted at Microbiology Department of Combined Military Hospital, Multan which is a 1600 bedded teaching hospital receiving patients of all categories and ages. A total of 96 patient samples were included in the study through consecutive non-probability sampling technique from January 2024 to June 2024. Patients of both gender and of all ages with suspected pulmonary TB and extrapulmonary tuberculosis were included in the study. Samples from diagnosed cases of Tuberculosis and those on ant tuberculosis therapy were excluded from study. Early morning sputum sample were collected in a sterile bottle after rinsing mouth thoroughly with water to avoid contamination. Other samples included CSF, bronchioalveolar lavage, pleural effusion and body tissues which were received at Microbiology Department. Collected sample were sent for MTB GeneXpert and assay was performed as per recommended guidelines. GeneXpert positive with Rifampicin resistance were labelled as detected and negative as not detected. 2ml sample

RESULTS:

A total of 96 samples were processed through MTB GeneXpert.

Figure:Distribution according to gender

31, 32% 65, 68%

■ Males
■ Females

The figure shows the sample distribution according to gender. This shows that out of 96 samples, 31 samples were collected from females and 65 samples were from male patients.

Table: Detection Ratio Rifampicin Resistance By GeneXpert (N=96)

SAMPLES	Total	POSITIVE FOR TUBERCULOSIS	RIF RESISTANCE DETECTION Ratio
SPUTUM	396	73	5
CSF	39	3	1
PLEURAL EFFUSION	43	8	1
BRONCHIOALVEOLAR LAVAGE	28	10	2
PUS	17	2	0
TOTAL	523	96 (18.3%)	9 (9.3%)

Chi square 4.7 p value 0.31

Table shows the frequency of Rifampicin resistance detection of various samples by GeneXpert. Out of total 96 samples, 73 samples of sputum were positive for Mycobacterium tuberculosis and 23 samples from other body fluids such as CSF, Pleural effusion, bronchioalveolar lavage and pus were positive for mycobacterium. The detection ratio of Rifampicin resistance by GeneXpert was 9.3 %.

DISCUSSION:

Due to the emergence of drug resistant tuberculosis it has become global challenge to control and prevent tuberculosis (WHO, 2022). Utilization of molecular based diagnostic methods with detection of multi drug resistant tuberculosis can reduce the turnaround time required to diagnose cases from weeks to hours (Ismail et al., 2016). To minimize the spread of rifampicin resistant tuberculosis strains, early detection of mycobacterium should be strengthened for the management of affected patients (Wasihun et al., 2020).

GeneXpert Assay is a rapid and reliable technique for detection of Mycobacterium and Rifampicin resistance which not only reduces the mortality but is also crucially important for starting proper treatment (Elbrolosy et al.,2021).

The current study was conducted to determine the detection ratio of Rifampicin resistant tuberculosis among various pulmonary and extra-pulmonary clinical specimens. The samples were categorized further into sputum, CSF, Pleural effusion,

bronchioalveolar lavage and pus specimens. Of all specimens, frequency of tuberculosis was found to be 18.3% and frequency of Rifampicin resistance as 9.3 %. In similar study conducted in Ethiopia, the prevalence of rifampicin resistant tuberculosis varied from 2.3% to 34.3% across studies in the country (Demelash et al., 2023). Overall MDR rate was found to be 2.7% in a study conducted by Mahmood et al in Hazaro District of Pakistan in year 2024. The results of our study also show that extra-pulmonary samples were found to be positive more frequent than sputum samples which shows that tuberculosis in our country in not limited to lungs only but also presents with extra pulmonary manifestations. In this study GeneXpert proved to be good diagnostic tool not only for pulmonary but also extrapulmonary specimens. The results of this study is comparable to studies conducted at various parts of the country and region specifically and globe particularly where GeneXpert was used as diagnostic tool for various clinically tuberculosis suspected specimens (Sasikumar et al., 2020) (Chaudry et al 2021).

In conclusion, GeneXpert is an accurate and rapid tool for diagnosis of pulmonary and extrapulmonary tuberculosis and Rifampicin resistance. Tuberculosis endemic countries like Pakistan where MDR and XDR tuberculosis is prevalent at increased frequency, there is increased need for early, rapid and accurate diagnostics tools for tuberculosis diagnosis, treatment and follow up to end this endemic.

REFERENCES

1. Agrawal, M., Bajaj, A., Bhatia, V. and Dutt, S., 2016. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. *J. of cli & dia research: JCDR*, *10*(5), p.DC09.

2. Brown, S., Leavy, J.E. and Jancey, J., 2021. Implementation of GeneXpert for TB testing in low-and middle-income countries: a systematic review. *Global Health: Sci and Practice*, *9*(3), pp.698-710.

3. RAHMAN, N., TAJ, M.H., JAMI, A., QURESHI, A., SHAH, M.Z. and KHALIQ, N., Yield of Gene Expert and ZN Staing in diagnosis of Pulmonary and Extra Pulmonary Tuberculosis.

4. Khan, S.U., Rahman, H., Ayaz, S., Qasim, M., Jabbar, A., Khurshid, M., Hussain, M., Muhammad, N., Rehman, S.U. and Ali, N., 2016. GeneXpert assay for rapid detection of Mycobacterium tuberculosis complex in respiratory specimens from a high TB endemic area of Pakistan. *Microbial pathogenesis*, *95*, pp.82-85.

5. Shah, S.W.H., Ullah, R., Raja, S.K., Ahmed, J., Onaiz, S., khaliq Ansari, J., Naqvi, S. and Iqbal, Z., 2019. Diagnostic accuracy of

GeneXpert and sputum Zeil Nelson Staining in predicting tuberculosis taking Sputum Culture as Gold standard. *Pakistan Journal of Chest Medicine*, *25*(2), pp.68-73.

6. Elbrolosy, A.M., El Helbawy, R.H., Mansour, O.M. and Latif, R.A., 2021. Diagnostic utility of GeneXpert MTB/RIF assay versus conventional methods for diagnosis of pulmonary and extra-pulmonary tuberculosis. *BMC microbiology*, *21*, pp.1-10.

7. Sasikumar, C., Utpat, K., Desai, U. and Joshi, J., 2020. Role of GeneXpert in the diagnosis of mycobacterium tuberculosis. *Advances in Respiratory Medicine*, *88*(3), pp.183-188.

8. Chaudhary, R., Bhatta, S., Singh, A., Pradhan, M., Srivastava, B., Singh, Y.I., Sah, R., Fathah, Z., Mehta, R., Rabaan, A.A. and Rodriguez-Morales, A.J., 2021. Diagnostic performance of GeneXpert MTB/RIF assay compared to conventional Mycobacterium tuberculosis culture for diagnosis of pulmonary and extrapulmonary tuberculosis, Nepal. *Narra J*, *1*(2).

9. World Health Organization, 2022. *Global tuberculosis report 2021: supplementary material.* World Health Organization.

10. Ismail, N.A., Ismail, M.F., Noor, S.S.M. and Camalxaman, S.N., 2016. Genotypic detection of rpoB and katG gene mutations associated with rifampicin and isoniazid resistance in Mycobacterium tuberculosis isolates: A local scenario (Kelantan). *The Malaysian Journal of Medical Sciences: MJMS*, *23*(1), p.22.

11. Wasihun, A.G., Dejene, T.A. and Hailu, G.G., 2020. Frequency of MTB and rifampicin resistance MTB using Xpert-MTB/RIF assay among adult presumptive tuberculosis patients in Tigray, Northern Ethiopia: a cross sectional study. *PloS one*, *15*(11), p.e0240361.

12. Demelash, M., Nibret, E., Hailegebriel, T., Minichil, Z. and Mekonnen, D., 2023. Prevalence of rifampicin resistant pulmonary tuberculosis using geneXpert assay in Ethiopia, a systematic review and meta-analysis. *Heliyon*.

13. Mehmood, S., Pervaiz, F., Mahmood, H., Riaz, M., Khan, J. and Diba, F., 2024. GeneXpert Assay in Detection of Pulmonary Tuberculosis and Rifampicin Tuberculosis Resistant in Tehsil Hazro Population; A Cross Sectional Study. *Journal of Islamabad Medical & Dental College*, *13*(3), pp.473-479.