International Journal of Medical and Pharmaceutical Research
Website: https://ijmpr.in/ | Print ISSN: 2958-3675 | Online ISSN: 2958-3683
NLM ID: 9918523075206676
Volume: 4 Issue:5 (Sept-Oct 2023); Page No: 347-351

Original Article

IJMPR

Gene-Xpert: Cutting Edge Technology For Diagnosis of Extrapulmonary-Tuberculosis among Aerobically Sterile Pus & Tissue Samples of Tertiary Care Hospital, Ahmedabad

Dr. Prutha Contractor[1*]; Dr. Navin Shah[2]; Dr. Rina Chandravadiya[3]; Dr. Rachana Solanki[4]

[1] 2nd Year PG Resident, Department of Microbiology, Dr. M.K.Shah Medical College & Research Centre, Ahmedabad
[2] Professor & Head of Department, Department of Microbiology, Dr. M.K. Shah Medical College & Research centre, Ahmedabad.
[3] Senior Resident / Tutor, Department of Microbiology, Dr. M.K. Shah Medical College & Research centre, Ahmedabad.
[4] Professor, Department of Microbiology, Dr. M.K. Shah Medical College & Research centre, Ahmedabad.

ABSTRACT

Introduction: Diagnosis of Extrapulmonary TB (EPTB) remains a challenge as the number of Mycobacterium tuberculosis (MTB) bacilli present in tissues and pus samples from various sites of disease are often low & non-uniformly distributed. Molecular diagnostic methods like GeneXpert or Cartridge-based nucleic acid amplification test (CBNAAT) seems to hold the key to future of better and efficient diagnosis of Extrapulmonary Tuberculosis (EPTB) in pus and tissue samples as it simultaneously detects *M. tuberculosis* and Rifampicin resistance in samples.

Material & Methods : A Retrospective cross sectional study was carried out in the Department of Microbiology. This study includes pus and tissue samples from November 2021 to December 2022 – 14 months. All pus and tissue samples were subjected to aerobic culture, simultaneously Gram stain, ZN stain, Modified ZN stain, KOH mount were performed among them. All samples that met the inclusion criteria which were sterile and smear negative (Gram stain, ZN stain, Modified ZN, KOH mount) were subjected to clinical evaluation. In these cases, detailed clinical history for TB were taken and samples were subjected to GeneXpert. Later on data were analysed on the basis of clinically suspected and clinically not suspected for TB.

Inclusion criteria: Frank Pus sample > 3 ml, Tissue minimum 1 gm

Exclusion criteria: Blood-stained Pus, Pus samples < 3 ml, Pus swab, Tissue received in Formalin, Clinical history not available

Results: Total 387 samples (360 pus, 27 tissue) received for aerobic culture. Among them 198 samples (181 pus, 17 tissue) were aerobically sterile and smear negative (Gram stain, Zn stain, Modified ZN, KOH mount). Out of 181 pus samples, 47 samples (13.05%) met the inclusion criteria and were subjected to Gene-Xpert. Among these 15 positive samples, 11 samples (23.40%) were clinically suspected & 4 samples (8.51%) clinically not suspected were detected of M. Tuberculosis. Among 27 tissue samples, 17 sterile and smear negative samples were subjected to Gene-Xpert, among them only 2(11.76%) clinically not suspected samples of Tissue were detected for M. Tuberculosis.

Conclusion: Diagnosis of EPTB is very important as pus and tissue are regarded as precious samples which cannot be repeatedly retrieved. Our data suggest use of GeneXpert for diagnosis of EPTB in smear negative, aerobically sterile pus & tissue samples.

Key Words: *Gene-Xpert, Extrapulmonary-Tuberculosis, CBNAAT*

***Corresponding Author**
Dr. Prutha Contractor
2nd Year PG Resident, Department of Microbiology, Dr. M.K.Shah Medical College & Research Centre, Ahmedabad

INTRODUCTION :

Tuberculosis (TB) remains a difficult challenge to undertake due to inadequate diagnostic assays. Extrapulmonary tuberculosis (EPTB) refers to TB in the body other than the lungs (e.g. meninges, lymph nodes, pleura, abdomen, genitourinary tract, skin, joints, and bones).[1]

EPTB is estimated to account for 8–24% (15% of the 7.0 million incident cases on average) of all TB infections worldwide.[2] In India, 15 to 20% of TB cases are estimated to be cases of EPTB, which affects mainly the lymph nodes, meninges, kidney, spine, and growing ends of the bones.[3] EPTB is responsible for significant morbidity and mortality, often due to subclinical or nonspecific symptoms that delay diagnosis and treatment.[4]

Diagnosis of Extrapulmonary TB (EPTB) remains a challenge as the number of Mycobacterium tuberculosis (MTB) bacilli present in tissues and pus samples from various sites of disease are often low & non-uniformly distributed. Also clinical specimens from deep-seated organs and lesion may be difficult to obtain. Histology is time-consuming and bewildering in establishing a diagnosis of TB with high specificity. ZN staining is often negative and when mycobacteria are seen, it is impossible to distinguish MTB from nontuberculous mycobacterial disease. Poor performance of conventional microbiological techniques in EPTB all contribute to challenges in diagnosing EPTB.[5]

CBNAAT is cartridge based nucleic acid amplification test with a well-established role in diagnosis of pulmonary tuberculosis (PTB).[4]This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hour.[5] The test isolates Mycobacterium genome from captured bacteria and amplifies DNA (DeoxyNucleic Acid) using polymerised chain reaction. GeneXpert test identifies relevant 81bp (base pair) fragment of MTB rpoB gene using flourescent probes called molecular beacons. GeneXpert assay detects MTB by polymerase chain reaction (PCR) amplification of rpoB gene and determination of rifampicin resistance by subsequent probing of this region for mutations that are associated with rifampicin resistance. Turnaround time of the test is 90 mins. GeneXpert requires approximately 130 bacilli per ml of sputum for positive result.[6]

In 2013, WHO formulated new guidelines, advocating the use of Xpert MTB/Rif assay for the diagnosis of EPTB. Several studies have showed successful use of GeneXpert test on extrapulmonary samples with high sensitivity and specificity.[7] Hence we decided to study and analyse its diagnostic accuracy in sterile pus and tissue samples for Extrapulmonary TB.

Early determination of rifampicin sensitivity is important for the timely detection of multidrug resistant tuberculosis and timely initiation of appropriate therapy in order to reduce the risk of morbidity & mortality. Gene-Xpert has a pooled sensitivity of 95% (95% CI 90-97%) and pooled specificity of 98% (95% CI 97-99%) for respiratory samples.[8]In Extrapulmonary samples (pus, tissue), Gene-Xpert has sensitivity of 86.4% (95% CI 69.28-96.24%) and specificity of 100% (95% CI 92.89%-100%).[20]

METHODOLOGY :

Material & Methods:

A Retrospective cross sectional study was carried out in the Department of Microbiology. This study includes pus and tissue samples from November 2021 to December 2022 – 14 months. All pus and tissue samples were subjected to aerobic culture, simultaneously Gram stain, ZN stain, Modified ZN stain, KOH mount were performed among them. All samples that met the inclusion criteria which were sterile and smear negative (Gram stain, ZN stain, Modified ZN, KOH mount) were subjected to clinical evaluation. In these cases, detailed clinical history for TB were taken and samples were subjected to GeneXpert. Later on data were analysed on the basis of clinically suspected and clinically not suspected for TB.

Inclusion criteria: Frank Pus sample > 3 ml, Tissue minimum 1 gm
Exclusion criteria: Blood-stained Pus, Pus samples < 3 ml, Pus swab, Tissue received in Formalin, Clinical history not available

Analysis of samples by Xpert MTB/RIFassay : The assay was performed using version 4 cartridges according to the manufacturers' recommendations. Samples were processed as per Gene-Xpert Module (6).The sample was diluted with three times the reagent, incubated at room temperature and loaded into the cartridge for automated analysis with results in 100 min. Detection of mycobacteria and rifampicin resistance was carried-out in the same setting.

RESULTS:

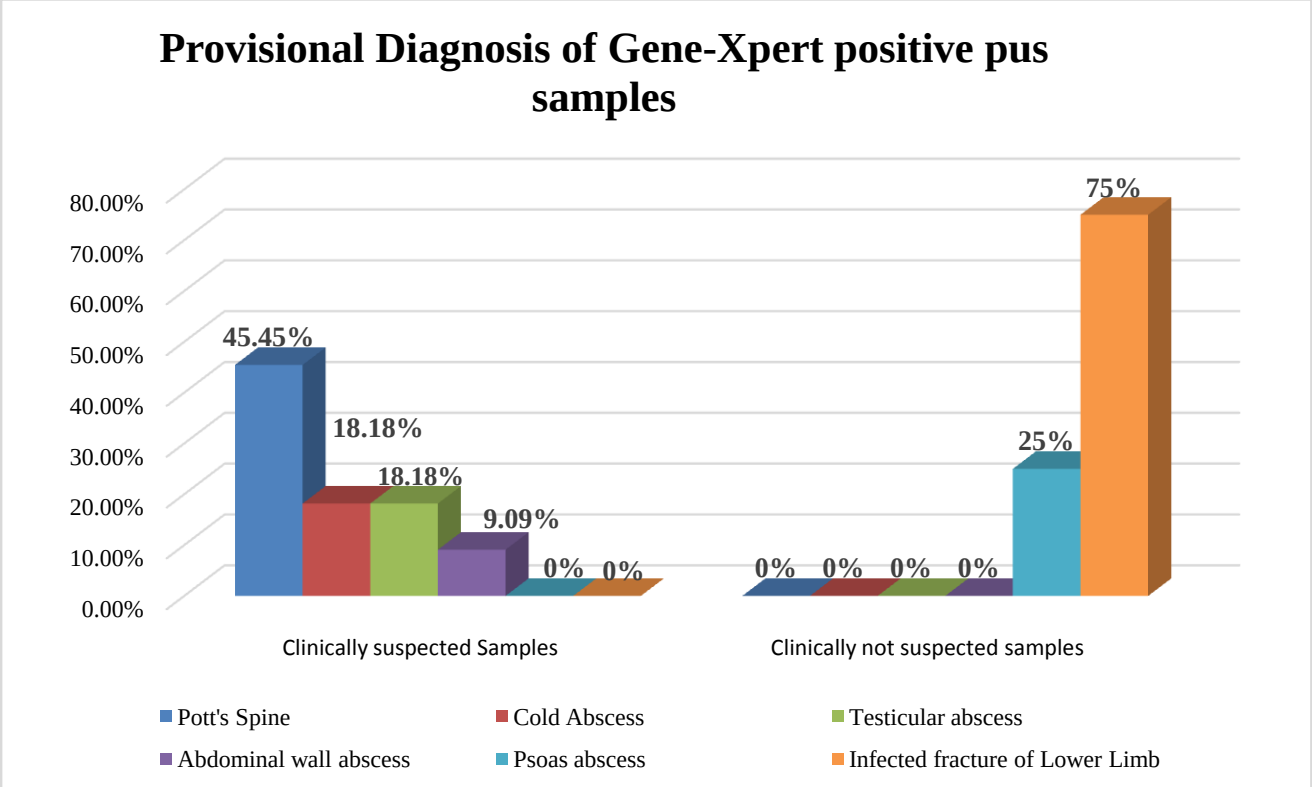
Total 387 samples (360 pus, 27 tissue) received for aerobic culture. Among them 198 samples (181 pus, 17 tissue) were aerobically sterile and smear negative (Gram stain, Zn stain, Modified ZN, KOH mount). Out of 181 pus samples, 47 samples (13.05%) met the inclusion criteria and were subjected to Gene-Xpert.

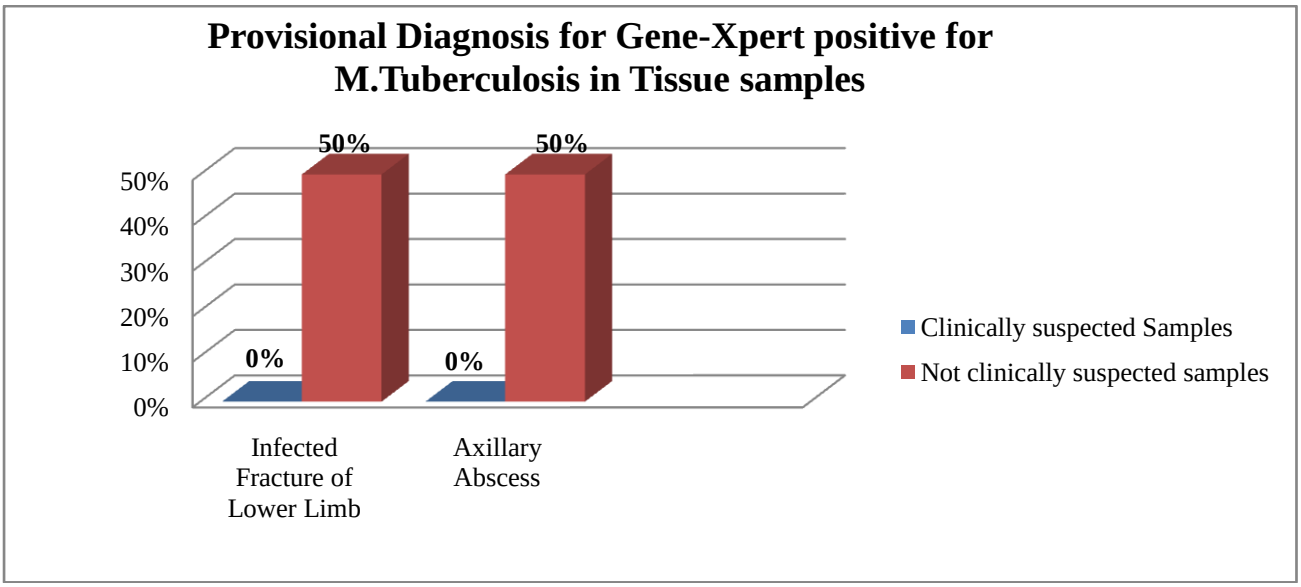
Pus samples subjected to Gene-Xpert were collected from various sites (Lower spine, hip, fracture sites, abscess, surgical site infection from thigh, forearm, lymphadenopathy).

Type of sample	Total number of samples	Number of aerobically sterile and smear negative samples	Number of samples subjected to Gene-Xpert	Number of samples in which Gene-Xpert positive for M.Tuberculosis
Pus	360	181 (50.27%)	47 (13.05%)	15 (31.91%)
Tissue	27	17 (62.96%)	14 (51.85%)	2 (11.76%)

Among 27 tissue samples, collected from various sites like (fracture sites, bronchial site, hip, swelling over lower back,gluteal abscess, surgical site infection of thigh, tibia, ankle, forearm), 17 tissue samples were sterile and smear negative with provisional diagnosis like (Hip Avascular necrosis, Lumbar spine stenosis, Gluteal abscess, fracture sites of lower limb). These 17 sterile and smear negative samples were subjected to Gene-Xpert, among them only 2(11.76%) clinically not suspected samples of Tissue were detected for M. tuberculosis with provisional diagnosis of Infected Upper tibia fracture and Axillary abscess.

Number of aerobically sterile and smear negative samples	Total samples subjected to Gene-Xpert	Total clinically suspected samples subjected to Gene-Xpert	Gene-Xpert positive for M.Tuberculosis in clinically suspected samples	Total clinically not suspected samples subjected to Gene-Xpert	Gene-Xpert positive for M.Tuberculosis in clinically not suspected samples
Pus (181)	47 (25.96%)	11 (6.07%)	11 (6.07%)	36 (19.88%)	4 (2.21%)
Tissue (17)	14 (82.35%)	2 (11.76%)	0	12 (70.58%)	2 (11.76%)





All patients are on follow-up who were not clinically suspected and are improving on AKT.

DISCUSSION :

Laboratory confirmed diagnosis of tuberculosis is pivotal for management of disease and reduce the transmission of infection. Improved detection of tuberculosis is considered a priority by World health organization.[8]

Introducing the Xpert MTB/RIF assay early in the diagnostic workflow can potentially improve detection and shorten turn-around time in the laboratory. Recent reports suggest that Xpert MTB/RIF assay increases the rate of detection of RIF resistance, decreases unnecessary empiric treatment among smear-negative extrapulmonary TB and increases the early initiation of second-line drugs treatment.[14]

In our study, among 387 extrapulmonary samples, Gene-Xpert was positive for M.tuberculosis in 17 samples (4.39%) which was lower when compared to Avashia S et al[11] which shows among total 300 EPTB samples , Gene-Xpert was positive for M.tuberculosis in 71 samples (23.67%).

In this study, among total 198 aerobically sterile and smear negative samples, Gene-Xpert was positive for M.tuberculosis in 17 samples (8.58%) which was higher when compared to similar study Zahoor D et al[16] which shows among total 224 aerobically sterile and smear negative samples, Gen-Xpert was positive for M.tuberculosis in 2 samples (0.89%).

In our study, among 17 EPTB samples in which M.tuberculosis was detected 5 (29.41%) were TB sine cases & 5 (29.41%) were other bone TB cases which was higher when compared to Chinnu Sasikumar et al[12] which shows out of the 103 extrapulmonary TB cases; 11 were TB spine cases (10.67%), 9 were other bone TB cases (8.73%).

Our study highlighted that CBNAAT can be a faster alternative to time taking methods like culture DST and at the same time a more efficient alternative to other rapid methods like AFB smear examination in the diagnosis of EPTB. With our study, we conclude that CBNAAT is more effective as compared to AFB smear in the diagnosis of EPTB cases and CBNAAT should be routinely utilized for rapid diagnosis of EPTB along with other conventional methods like AFB smear examination and culture DST for better overall results in the diagnosis of EPTB.[13]

CONCLUSION :

Diagnosis of EPTB is very important and requires early diagnosis and immediate treatment. Pus and tissue are regarded as precious samples which cannot be repeatedly retrieved. Gene-Xpert's sensitivity scored twice as high in comparison with microscopy thus doubling the proportion of rapid diagnoses, with important rebound on the patients' outcome.[19]

Gene-Xpert allows accurate and early diagnosis of TB infection and the shorter turnaround time provides an opportunity for timely therapeutic intervention, which is beneficial not only for the patient but also for any possible contacts.[17] Our data suggest use of Gene-Xpert for diagnosis of EPTB in smear negative, aerobically sterile pus & tissue samples.

REFERENCES :

1. Diagnosis and Treatment of Extrapulmonary TuberculosisJi Yeon Lee, M.D
2. Xpert MTB/RIF assay for diagnosis of extrapulmonary tuberculosis in children: a systematic review and meta-analysisYoung Seok Seo, Ji-Man Kang, Dong Soo Kim, and Jong GyunAhn
3. Xpert MTB/RIF: a New Pillar in Diagnosis of Extrapulmonary Tuberculosis?
4. Viral Vadwai,[1] Catharina Boehme,[2] Pamela Nabeta,[2] Anjali Shetty,[1] David Alland,[3] and Camilla Rodrigues Effectiveness of CBNAAT in the diagnosis of extrapulmonary tuberculosis ShivprasadKasat, MahendraBiradar*, Ashish Deshmukh, Sunil Jadhav, Hafiz Deshmukh
5. Diagnosis of extrapulmonary tuberculosis using the Xpert® MTB/RIF assay Stephen D Lawn[*,1,2] and Alimuddin I Zumla[3,4]
6. 22. Indian J Otolaryngol Head Neck Surg (Oct–Dec 2018) 70(4):572–577
7. World Health Organization. Global Tuberculosis Report. Geneva, Switzerland: World Health Organization, 2017.
8. World Health Organization. Implementing tuberculosis diagnostics: A policy framework. Geneva World Heal Organ. 2015;39. Available from:http://www.who.int/tb/publications/implementing_TB_diagnostics/en/.
9. World Health Organization. Global Tuberculosis Report. Geneva, Switzerland: World Health Organization, 2017.
10. Sharma SK, Mohan A. Tuberculosis: From an incurable scourge to a curable disease - journey over a millennium. Ind J Med Res. 2013;137:455-93.
11. Avashia S et al, Comparision of conventional methods with gene xpertmtb/rif assay for rapid detection of mycobacterium tuberculosis and rifampicin resistance in extra-pulmonary samples
12. Chinnu Sasikumar et al., The role of GeneXpert in the diagnosis of Mycobacterium tuberculosis
13. World Health Organization. Rapid implementation of the Xpert MTB/RIF diagnostic test. Technical and operational "How to" practical considerations. World Heal Organ Doc. 2011;WHO/HTM/TB:1-34.
14. Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, et al. Advances in tuberculosis diagnostics: The Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis. 2013;13(4):349-61.
15. Central TB Division. Directorate General of Health Services Ministry of Health and Family Welfare. TB India 2016. Revised National TB Control Programme. Technical and operational guidlines for tuberculosis control in India. 2016:144.
16. Zahoor D et al. Int J Res Med Sci. 2018 May;6(5):1756-1760
17. Habous, et al.: Evaluate GeneXpert for detecting MTB in nonrespiratory specimens
18. Malbruny B, Le Marrec G, Courageux K, Leclercq R, Cattoir V. Rapid and efficient detection of Mycobacterium tuberculosis in respiratory and nonrespiratory samples. Int J Tuberc Lung Dis. 2011;15(4):553-5.
19. E. TORTOLI ET AL. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur Respir J 2012; 40: 442–447
20. Mukherjee et al.: CBNAAT in extrapulmonary tuberculosis