

Original Research Article

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Evolution of Genexpert MTB/RIF Assay for Rapid Diagnosis of Tuberculosis in Extra Pulmonary Sample of Suspected Case of Tuberculosis in Tertiary Care Hospital, Jamnagar, Gujarat (India)

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ABSTRACT

Tuberculosis is caused by various strains of Mycobacteria usually Mycobacterium tuberculosis. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air. In 15-20% of active cases, the infection spreads outside the lungs, causing other kinds of TB. These are collectively denoted as “Extra pulmonary Tuberculosis” extra pulmonary TB occurs more commonly in immune suppressed persons and young children. This study was conducted in tertiary care hospital, Jamnagar. Total 230 Extra Pulmonary (Pus, Body Fluid, Abscess, CSF, Lymph nodes, Biopsy, Urine, BAL, Tissue) Suspected case of MTB samples were received during the study period. Out of total 230 Extra pulmonary samples, in 64(27.82%) samples Mycobacterium Tuberculosis Bacilli (MTB) were detected by GeneXpert MTB/RIF Assay. Out of 64 positive extra pulmonary MTB cases, 32(50%) were belongs to male gender and 32(50%) were belongs to female gender, highest numbers of MTB positive cases were found in age group of 11-20 years which were 18(28.1%) followed by 21-30 years which were 15(23.4%). The study revealed the GeneXpert MTB/RIF Assay test offers a potential solution for improving early MTB diagnosis.

Keywords

Mycobacterium tuberculosis, GeneXpert MTB/RIF Assay, Extra pulmonary

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Introduction

Tuberculosis is a disease caused by bacteria that are spread from person to person through the air. Tuberculosis usually affects the lungs, but it can also affect other parts of body, such as the brain, the kidneys or the spine. In most case, TB is treatable and curable; however, person with TB can die if they do not get proper treatment. Tuberculosis is a worldwide public health problem despite the highly

effective drugs and vaccine are available making tuberculosis a preventable and curable disease^[3].

Roughly one third of the world's population has been infected with M. Tuberculosis^[5], with new infections occurring in about 1% of the population each year^[6].

Most infections with M. tuberculosis do not cause TB disease^[7], and 90-95% of infections

remain asymptomatic ^[8]. In 2012, an estimated 8.6 million chronic cases were active ^[9]. In 2012, 8.8 millions new case of Tb were diagnosed, and 1.20-1.45 million death occurred, most of these occurring in developing countries ^[10, 11]. Of these 1.45 million deaths about 0.35 million occur in those also infected with HIV ^[12].

Tuberculosis is the second most common cause of death from infectious disease (after those due to HIV/AIDS) ^[13]. The total number of tuberculosis cases has been decreasing since 2005, while new cases have decreased since 2002 ^[10]. The number of new cases has declined by 17% between 2004 and 2014 ^[14]. Tuberculosis is more common in developing countries about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5-10% of the US population test positive ^[15].

In 15-20% of active cases, the infection spreads outside the lungs, causing other kinds of TB ^[4]. These are collectively denoted as “Extra pulmonary Tuberculosis” extra pulmonary TB occurs more commonly in immune suppressed persons and young children. In those with HIV, this occurs in more than 50% of cases ^[17].

The Xpert MTB/RIF is a cartridge-based nucleic acid amplification test (NAAT) for simultaneous rapid tuberculosis diagnosis and rapid antibiotic sensitivity test. It is an automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) DNA and resistance to rifampicin (RIF). It was co-developed by the laboratory of Professor David Alland at the University of Medicine and Dentistry of New Jersey (UMDNJ) ^[18]. Cepheid Inc. and Foundation for Innovative New Diagnostics, with additional financial support from the US National Institutes of Health (NIH).

In December 2010, the World Health Organization (WHO) endorsed the Xpert MTB/RIF for use in TB endemic countries ^[19]. This followed 18 months of assessment of its field effectiveness in TB, MDR-TB and TB/HIV co-infection ^[20]. The CDC said in 2015^[21] that the Xpert MTB/RIF test was "revolutionizing tuberculosis (TB) control by contributing to the rapid diagnosis of TB disease and drug resistance. The test simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampicin (RIF) in less than 2 hours.

The Xpert MTB/RIF detects DNA sequences specific for *Mycobacterium tuberculosis* and rifampicin resistance by polymerase chain reaction^{[22][23]} It is based on the Cepheid GeneXpert system, a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT). The Xpert MTB/RIF purifies and concentrates *Mycobacterium tuberculosis* bacilli from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR. The process identifies all the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (*rpoB*) gene in the *Mycobacterium tuberculosis* genome in a real time format using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very little technical training required to operate ^[24].

Materials and Methods

This study was conducted in the tertiary care hospital, from August 2017 to September 2018. All samples were collected after detail review of clinical history and laboratory findings. Different Extra pulmonary samples (Pus, Body Fluid, Abscess, CSF, Lymph nodes, Biopsy, Urine, BAL, Tissue) collected

and transported from various centers to TB culture – DST laboratory with cold chain maintained. Total suspected samples received during study period and proceeded for Xpert MTB/RIF Assay.

The GeneXpert Dx System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. Xpert MTB/RIF includes reagents for the detection of tuberculosis and RIF resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair “core” region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

Sample preparation

Lymph nodes and other tissues (for xpert MTB/RIF only)

Using sterile pair of forceps and scissors, cut the tissue specimen into small pieces in a sterile mortar (or homogenizer or tissue grinder). Add approximately 2 ml of sterile phosphate buffer (PBS). Grind the solution of

tissue and PBS using a mortar and pestle (or homogenizer or tissue grinder) until a homogeneous suspension has been obtained. Use a transfer pipette to transfer approximately 0.7 ml of the homogenized tissue specimen to a sterile, conical screw-capped tube. NOTE: Avoid transferring any clumps of tissue that have not been properly homogenized. Use a transfer pipette to add a double volume of the Xpert MTB/RIF Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue. Vigorously shake the tube 10 to 20 times or vortex for at least 10 seconds. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously again for another 10–20 times or vortex for at least 10 seconds. Incubate the specimen at room temperature for an additional 5 minutes. Using a fresh transfer pipette, transfer 2 ml of the processed sample to the Xpert MTB/RIF cartridge. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

CSF

The preferred processing method for CSF in Xpert MTB/RIF depends on the volume of specimen available for testing. NOTE: Blood-stained and xanthochromic CSF specimens may cause false-negative results from Xpert MTB/RIF.

If there is more than 5 ml of CSF: transfer the entire specimen to a conical centrifuge tube, and concentrate the specimen at 3000 g for 15 minutes. Carefully pour off the supernatant through a funnel into a discard can containing 5% phenol or other mycobacterial disinfectant. Resuspend the deposit to a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent. Label an Xpert/MTB/RIF cartridge with the specimen's identification number. Using a fresh transfer pipette, transfer 2 ml of the concentrated CSF specimen to the Xpert MTB/RIF cartridge.

Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

If there is 1–5 ml of CSF: Add an equal volume of sample reagent to the CSF. Add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

If there is 0.1–1ml of CSF: Resuspend the CSF to a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent. Add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

If there is less than 0.1 ml: This is an insufficient sample for testing using the Xpert MTB/RIF assay.

Preparing the cartridge

Start the test within 30 minutes of adding the sample to the cartridge. Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark. Open the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge. Be sure to load the cartridge into the GeneXpert Dx instrument and start the test within 30 minutes of preparing the cartridge.

Interpretation of results

The results are interpreted by the GeneXpert DX System from measured fluorescent

signals and embedded calculation algorithms and will be displayed in the “View Results” window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.

Results and Discussion

This study was conducted in tertiary care Hospital from August 2017 to September 2018. Total 230 Extra Pulmonary (Pus, Body Fluid, Abscess, CSF, Lymph nodes, Biopsy, Urine, BAL, Tissue) Suspected case of MTB samples were received during the study period. The table.1 shows prevalence of MTB positive extra pulmonary samples. Out of total 230 Extra pulmonary samples, in 64 samples Mycobacterium Tuberculosis Bacilli (MTB) were detected by GeneXpert MTB/RIF Assay. So, prevalence of MTB positive extra pulmonary samples was 27.82%. (Table 1–5)

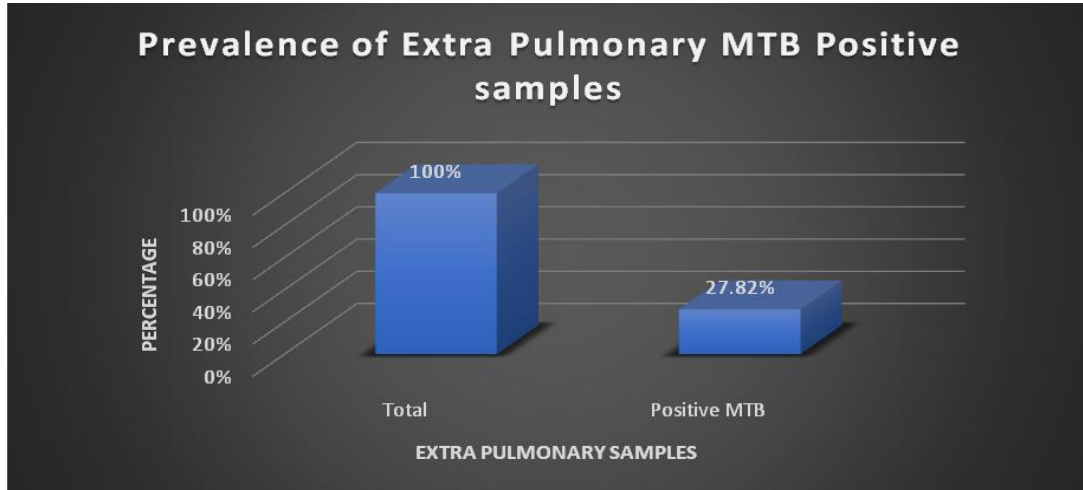
In conclusion, there are many conventional techniques available for diagnosis of tuberculosis now days which are microscopy, solid culture, liquid culture, line probe assay, but this study shows that the Genexpert/RIF Assay test helpful to epidemiological purpose to detect extra pulmonary tuberculosis.

The Genexpert/RIF Assay test offers a potential solution for improving early MTB diagnosis. The Genexpert/RIF is automated cartridge based nucleic acid amplification test, easy for use in peripheral labs and clinics by unskilled personnel. The use of Xpert is improve the diagnosis of Extra pulmonary TB compared to microscopy because tubercle bacilli load not enough for detection by smear microscopy.

Table.1 Shows prevalence of MTB positive extra pulmonary samples

Sr. No.	Total Extrapulmonary samples	MTB Detected	Prevalence
1.	230	64	27.82%

Chart-1



From which all 64 Extra pulmonary MTB positive samples were sensitive for Rifampicin.

Table.2 shows sex wise distribution of positive Extra pulmonary MTB samples

Sr. No.	Gender	Positive for MTB	Percentage
1.	Male	32	50%
2.	Female	32	50%
	Total	64	100%

Out of 64 positive extra pulmonary MTB cases, 32(50%) were belongs to male gender and 32(50%) were belongs to female gender.

Chart-2

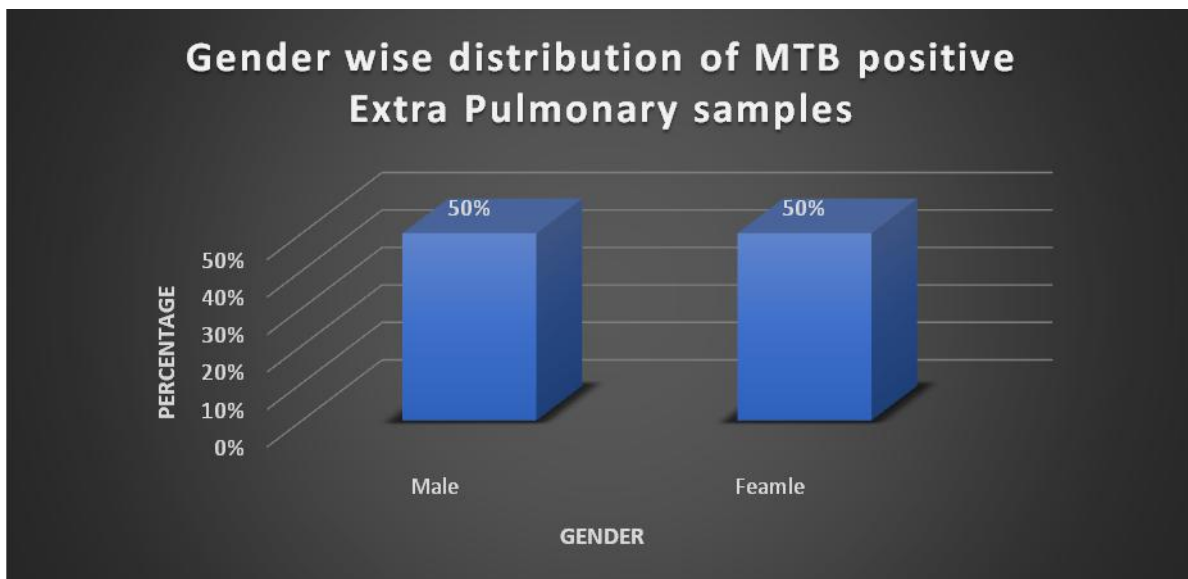


Table.3 shows Age wise distribution of Extra pulmonary samples MTB positive of patients of different age group

Sr. No.	Age (In Years)	Total MTB Positive	Percentage
1.	0-10	03	4.7%
2.	11-20	18	28.1%
3.	21-30	15	23.4%
4.	31-40	12	18.8%
5.	41-50	07	10.9%
6.	51-60	05	7.8%
7.	61-70	04	6.3%
8.	>70	00	0%
	Total	64	100%

Out of 64 Extra pulmonary MTB positive cases, highest numbers of MTB positive cases were found in age group of 11-20 years which were 18(28.1%) followed by 21-30 years which were 15(23.4%).

Chart-3

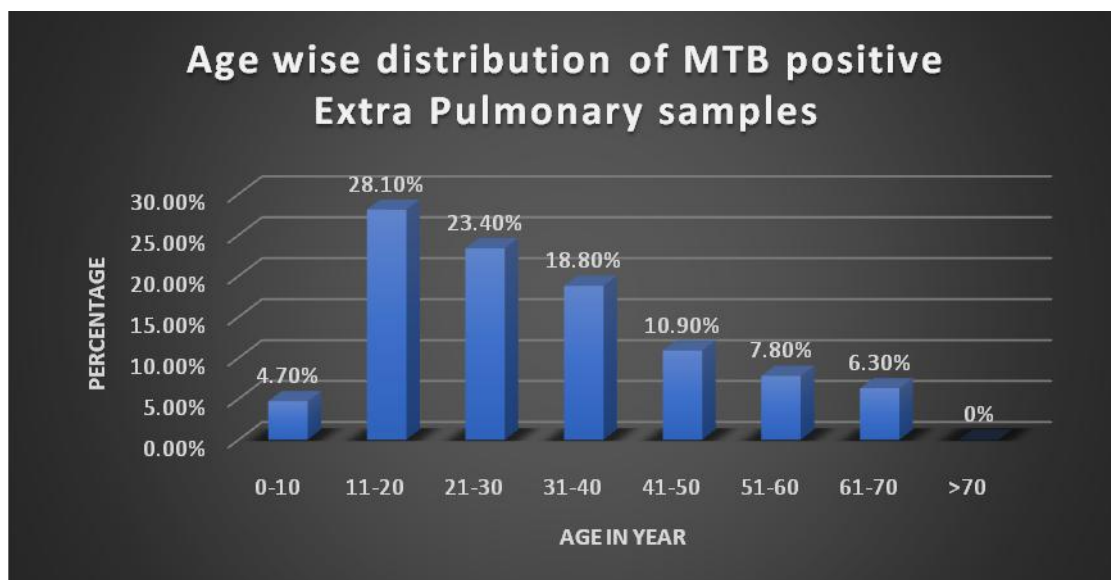


Table.4 Comparison of Prevalence of MTB positive extra pulmonary samples with different study

Sr. No	Study	Study Period (Year)	Prevalence
1.	Present Study	2017-18	27.82%
2.	Dhanya <i>et al.</i> , ^[25]	2016-18	14.39%
3.	Lesley Erica <i>et al.</i> , ^[16]	2012-13	22%

In present study, the prevalence of MTB positive cases in Extra pulmonary samples were 27.82% which compared with study of Lesley Erica *et al*^[16] in which prevalence of MTB positive cases was 22% and study of Dhanya *et al*^[25] prevalence of MTB positive cases was 14.39%.

Table.5 Gender wise comparison of Extra pulmonary MTB positive case with different study

Sr. No	Study	Male	Female
1.	Present Study	32/125 (25.6%)	32/105(30.48%)
2.	Dhanya ^[25]	16/157(10.19%)	17/100 (17%)

In present study, the gender wise distribution of MTB positive cases in Extra pulmonary samples were 25.6% and 30.48% in male and female respectively which compared with study of Dhanya et al ^[25] prevalence in male and female patients were 10.19% and 17% respectively.

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