E-ISSN:2349-3275
P-ISSN:2349-5502
Research Article
Tuberculosis

Biomedical Review-Journal of Basic and Applied Medical Science
2017 Volume 4 Number 1

Publisher
Biomedical Review
www.medresearch.in

Role of GeneXpert in early diagnosis of Pulmonary tuberculosis

Madhu S.[1], Sudhindra K.[2*], Sumanta A.[3], Sangolli B.[4], Ranganath R.[5]

[1] S Madhu, Assistant Professor, Department of General Medicine, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India.

[2*] K.S Sudhindra, Associate professor, Department of Microbiology, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India.

[3] A Sumanta, Assistant Professor, Department of Microbiology, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India.

[4] Basavaraj Sangolli, Assistant Professor, Department of Pulmonary Medicine, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India.

[5] R Ranganath, District Tuberculosis Officer, Department of Tuberculosis, District General Hospital, Chitradurga, Karnataka, India.

Background: The prevalence of tuberculosis infection is on the rise. There is an urgent need for rapid diagnosis of these cases for prompt initiation of treatment. It would be better if we can detect drug resistance in these cases before starting treatment. **Aim:** to evaluate the role of GeneXpert in the diagnosis of clinically suspected tuberculosis and to detect rifampicin resistance in these cases. **Materials and Methods:** A total of 1497 samples from April 2016 to March 2017, from all clinically suspected patients with support of either laboratory or radiological evidence from our teaching hospital and district general hospital were studied. **Results:** out of 1497 cases, 266 were found to be positive for MTB and 09 MTB positives cases were found to be resistant to rifampicin. **Conclusions:** GeneXpert was found to be simple, rapid and more effective method for detection of MTB cases and also the drug resistance could be detected simultaneously, thus reducing the time taken for initiation of treatment

Keywords: Tuberculosis, GeneXpert, Rifampicin resistance

Corresponding Author	How to Cite this Article	To Browse
K.S Sudhindra, Associate professor, Department of Microbiology, Basaveshwara Medical College & Hospital, Chitradurga, Karnataka, India. Email: dr_sudhindraks@yahoo.co.in	S Madhu, K.S Sudhindra, A Sumanta, Basavaraj Sangolli, R Ranganath, Role of GeneXpert in early diagnosis of Pulmonary tuberculosis. Biomed Rev J Basic Appl Med Sci. 2017;4(1):206-209. Available From https://www.biomedicalreview.in/role-of-genexpert-in-early-diagnosis-of-pulmonary-tuberculosis-research-article	

Manuscript Received	Review Round 1	Review Round 2	Review Round 3	Accepted
2017-06-07	2017-06-09	2017-06-16	2017-06-23	2017-06-30

Conflict of Interest	Funding	Ethical Approval	Plagiarism X-checker	Note
Nil	Nil	Yes	16%	

Introduction

Approximately one third of the world's population has been infected with M.tuberculosis (MTB) and is at risk of developing the disease later in life. The killer disease thus claims more than five thousand deaths everyday i.e., one every fifteen seconds [1]. Despite many advances in its diagnosis and treatment, problem of tuberculosis is on the rise, both globally and in India. The most effective control measure to check the spread of tuberculosis is to detect early to treat optimally at the earliest.

Although Ziehl-Neelsen (ZN) stain - smear microscopy is most commonly employed for early detection, it is rather insensitive and fails to detect large no. of cases [2]. Conventional culture methods such as LowensteinJensen (LJ) medium culture require 4-8 weeks for isolation plus additional 1-2 weeks for speciation. Such a prolonged turnaround time is unacceptable for both medical and epidemiological purpose [3]. Cartridge based nucleic acid amplification techniques (CBNAAT) such as GeneXpert have been recently recommended by World Health Organization and introduced in the diagnostic algorithms. This GeneXpert, also known as Xpert MTB/ RIF assay makes use of five different molecular beacons and each one of them being labelled with a differentially coloured flourophore and responding to a specific nucleic acid sequence within the rpoB gene of M. tuberculosis. Testing is carried out on the MTB/RIF test platform (GeneXpert, Cepheid), which integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection. The only manual step is the addition of a bactericidal buffer to sputum before transferring a defined volume to the cartridge.The MTB/RIF cartridge is then inserted into the GeneXpert device, which provides results within 2 hours [4]. This study was undertaken to evaluate the role of GeneXpert in the diagnosis of clinically suspected tuberculosis since it was introduced as a diagnostic tool in our district since last one year.

Materials and Methods

A total of 1497 samples from April 2016 to March 2017, from all clinically suspected patients with support of either laboratory or radiological evidence From our teaching hospital and district general hospital were studied. Demographic data and the necessary clinical details of the patients were collected from the patient's case sheets. Two sputum samples were collected in a sterile wide mouth container from all the patients, one being the spot sample and the other early morning sample. Informed consent was taken from all the patients. Both the samples were screened for presence of acid fast bacilli by conventional Zeihl Neelsen technique.

No sputum concentration method was used before smear preparation. One of the specimens was used for testing by GeneXpert. GeneXpert extraction reagent was mixed with the specimen in a ratio of 2:1 in a specimen container. The container was closed and shaken vigorously on a vortex mixer for 10 minutes. Then it was left at room temperature for 10 minutes after which it was once again shaken vigorously for 10 more minutes. The sample in the container was left to stand at room temperature for 10 minutes. Then the sample was checked to see if it completely fluid. If the sample was found to be still viscous, the shaking on vortex mixer was repeated further before it was taken for testing by GeneXpert. Finally 2ml of the processed specimen was taken in a sterile plastic disposable pipette and transferred to GeneXpert cartridge. The cartridge is loaded into the GeneXpert instrument, and an automatic process completes the remaining assay steps.The electronic results were sent directly from the GeneXpert test system to the central database. Lyophilized *Bacillus globigii* spores present in the assay cartridge serve as an internal process control. Assays that found to be negative for *M.tuberculosis* and also negative for *B.globigii* internal control are reported as invalid tests.

The test was repeated with the same mixture whenever an invalid result was found. If the repeat test with the same mixture was also found to show invalid result, a new specimen was requested from the patient and then processed. The repeat samples were not included for the study purpose to avoid duplication of the samples. Wherever possible, the patients were followed up to know their clinical status and the treatment outcome.

Results

Out of a total of 1497 cases, 199 (13.3%) (Table 1) were found out be positive for *M.tuberculosis*

By sputum smear examination whereas 266 (17.8%) (Table 2) cases were found positive by GeneXpert. So, GeneXpert was able to pick 67 which otherwise would have been missed by sputum smear examination alone. (Table 3) Out of the 266 cases found to be positive for MTB by GeneXpert, 09 (3.4%) cases were detected to be resistant to rifampicin (Table 4) suggestive of being multi drug resistant tuberculosis cases. 907 patients included in our study were found to be positive for HIV infection. Among these 102 (11.24%) were found to be positive for MTB by GeneXpert. However, only one HIV positive patient was found to be having MTB with rifampicin resistance (Table 5).

Table-1: Showing positivity rate of sputum smear microscopy.

No. of sputum samples tested	No. of Positives	No. of Negatives
1497	199 (13.3%)	1298 (86.7%)

Table-2: Showing positivity rate of GeneXpert

No. of sputum samples tested	No. of Positives	No. of Negatives
1497	266 (17.8%)	1231 (82.2%)

Table-3: Comparison of results of microscopy versus GeneXpert

	Smear positive	Smear negative	Total
GeneXpert positive	199	67	266
GeneXpert negative	00	1231	1231
Total	199	1298	1497

Table-4: Showing Rifampicin sensitivity results among MTB positive cases on GeneXpert

No. of MTB positive cases	Rifampicin Sensitive	Rifampicin Resistant
266	257 (96.6%)	09 (3.4%)

Table-5: MTB and HIV co-infection.

	HIV positive	HIV negative	Total
MTB positive, Rifampicin Sensitive	101	156	257
MTB positive, Rifampicin Resistant	01	08	09
MTB negative	805	426	1231
Total	907	590	1497

Discussion

Though India is the second-most populous country in the world, one fourth of the global incident TB cases occur in India annually. As per WHO Global TB Report, 2015, out of the estimated global annual incidence of 9.6 million TB cases, 2.2 million were estimated to have occurred in India [5]. It is estimated that about 40% of the Indian population is infected with TB bacteria, the Vast majority of whom have latent TB rather than TB disease. Sputum smear used to be most commonly used method for diagnosis of tuberculosis till very recently in most of the centres. Though better methods of diagnosis like culture by automated methods or diagnosis of tuberculosis by molecular methods were available, they were not being used in many cases owing to high cost per test or nonavailability at majority of the centres. With the continuation of using only microscopy as method for diagnosis of tuberculosis, there is every chance that few cases would be missed and these cases not put on antiTB treatment would continue to spread the disease among the community.

GeneXpert was installed at district hospital in our city with collaboration with RNTCP in March 2016. Since then all the clinically suspected tuberculosis cases are tested by this method. Among the 1497 clinically suspected cases tested for MTB, 199 (13.3%) were detected by microscopy alone and 266 (17.8%) cases turned out to be positive by GeneXpert. Different studies have given varying rates of detection by GeneXpert method. Singh et al [6] Bajrami et al [7] Ioannidis et al [8] and Dravid et al [9] have reported rate of detection to be 46.3%, 29.3%, 31.9% and 40.6% respectively. More studies will establish the sensitivity rate of this method to a certain extent. We would have missed 67 cases had we used only microscopy as a diagnostic method. Of the 266 cases found to be MTB positive, 09 (3.4%) were found to be resistant to rifampicin. Dravid et al [9] had found 17.1% rifampicin resistance in their study.These cases were presumptively considered as multi drug resistant tuberculosis (MDR-TB) cases and started on second line anti tubercular treatment after necessary pretreatment evaluation. However one more sample was collected from these cases and sent for confirmation of drug resistant status by line probe assays. All of them turned out to MDR-TB cases. Sputum smear examination for acid fast bacilli in HIV positive mostly turn to be negative even when they have active TB infection since in most of these cases, the bacterial load is too low to be picked on microscopy. In our study, out of 907 HIV positive cases, 102 (11.2%) were positive for MTB by GeneXpert. Arora et al [10] and Sethi et al [11] had found HIV- TB co-infection rates at 2.5% and 20.1% respectively. However only 66 (7.2%) were detected on microscopic examination. So 36 (35.2%) cases would have been missed by microscopy alone.

The main limitation in our study was that culture for mycobacteria was not done in these cases to rule false positive results. False-positive results are likely to be linked to the detection by GeneXpertof dead M. tuberculosis bacilli that would not be detected by culture, which is the present reference standard. The biggest advantage of GeneXpert is that of sample processing. The process is simplified into a very simple and single step where it liquefies and also inactivates the sputum resulting in the reduction of viable bacilli. Data from a recent study confirm that the MTB/RIF assay generates no infectious aerosols [12]. The simplicity and safety feature of this set up encourages us to use this as a rapid and highly sensitive method for detection of MTB cases as well as drug resistance in these cases without the need for sending the sample for higher centres and delaying the diagnosis. However the drawback of this set up is that it can process only four samples every two hours and also the cost can be too high for routine laboratories to afford.

Conclusion

GeneXpert was found to be simple, rapid and more effective method for detection of MTB cases and also the drug resistance could be detected simultaneously, thus reducing the time taken for initiation of treatment. Due to the economic constraints in resource limited settings and those laboratories receiving large numbers of samples, judicious use of GeneXpert is recommended.

Reference

1. Grange JM, Zumla AI. Tuberculosis. In: Manson's Tropical diseases. 22nd edition. Saunders, Elsevier; 2009.p.983-1038.

2. Hall GS. Primary processing of specimens and isolation and cultivation of mycobacteria. Clin Lab Med. 1996 Sep;16(3):551-67.

3. Park C, Hixon D, Furguson C. Rapid recovery of mycobacteria from clinical specimens using automated radiometric technique. Am J Clin Path 1984;81:341-5.

4. Piatek AS, Tyagi S, Pol AC, Telenti A, Miller LP, Kramer FR, Alland D. Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis. Nat Biotechnol. 1998 Apr;16(4):359-63.

5. Lienard J, Carlsson F. Murine Mycobacterium marinum Infection as a Model for Tuberculosis.Methods Mol Biol. 2017;1535:301-315.

6. Singh UB, Pandey P, Mehta G, Bhatnagar AK, Mohan A, Goyal V et al. Genotypic, phenotypic and clinical validation of GeneXpert in extra-pulmonary and Pulmonary tuberculosis in India. PLoS One 2016;11: e0149258.

7. Bajrami R, Mulliqi G, Kurti A, Lila G, Raka L. Comparison of GeneXpert MTB/RIF and conventional methods for the diagnosis of tuberculosis in Kosovo. J Infect Dev Ctries. 2016 Apr 28;10(4):418-22. doi: 10. 3855 /jidc.7569.

8. Ioannidis P, Papaventsis D, Karabela S, Nikolaou S, Panagi M, Raftopoulou E, et al. Cepheid GeneXpert MTB/RIF Assay for Mycobacterium tuberculosis detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. J Clin Microbiol 2011;49:3068–70.

9. Dravid MN, Shah P, Dange S, and Suryawanshi M. Rapid detection of Mycobacterium tuberculosis Cases by GeneXpert- 1 Year Retrospective Study. J Med Res Prac 2016;06:52-5.

10.Arora D, Jindal N, Bansal R, Arora S.Rapid and accurate detection of mycobacterium tuberculosis in sputum samples by cepheidXpert assay-a clinical study. J Clin Diagn Res 2015;9:DC03–DC05.

11. Sethi S, Mewara A, Dhatwalia SK, Singh H, Yadav R, Singh K, et al. Prevalence of multidrug resistance in Mycobacterium tuberculosis isolates from HIV seropositive and seronegative patients with pulmonary tuberculosis in north India. BMC Infect Dis 2013; 13:137.

12. Banada PP, Sivasubramani SK, Blakemore R, Boehme C, Perkins MD, Fennelly K, Alland D. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. J Clin Microbiol. 2010 Oct;48(10):3551-7. doi: 10.1128/JCM.01053-10. Epub 2010 Aug 18.