DETERMINANTS OF DOTS ADHERENCE AMONG CBNAAT POSITIVE TB PATIENTS REFERRED BY PRIVATE HEALTH FACILITIES OF BHUBANESWAR

By

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Admission No: 01MB/18

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A Thesis submitted to the Odisha University of Agriculture and Technology in partial fulfilment of the requirement for the degree of (*Masters of Science*) *in Microbiology*

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Reference No. :
Date : 16.07.20

CERTIFICATE-I

This is to certify that the thesis entitled **"Determinants of DOTS adherence among CBNAAT positive TB patients referred by private health facilities of Bhubaneswar"** submitted in partial fulfilment of the requirements for the award of the degree of **Masters of Science in Microbiology** to the **Odisha University of Agriculture and Technology**, Bhubaneswar, Odisha is a faithful record of bonafide and original research work carried out by ***Ms. Sumita Panigrahi*** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma. It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

(Dr. Jyotirmayee Turuk)
Supervisor

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CERTIFICATE-II

This is to certify that the thesis entitled **"Determinants of DOTS adherence among CBNAAT positive TB patients referred by private health facilities of Bhubaneswar"** submitted by the student bearing Admission No. 01MB/18 to the Odisha University of Agriculture and Technology, Bhubaneswar, in partial fulfillment of the requirements for the award of the degree of **Masters of Science in Microbiology**, has been approved by the student's advisory committee and the external examiner.

External Examiner

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DECLARATION

I do here by declare that the thesis entitled **"Determinants of DOTS adherence among CBNAAT positive TB patients referred by private health facilities of Bhubaneswar"** is a record of original research work conducted by me and that no part of the thesis has been submitted before for award of any other degree or diploma of any University.

Ms. Sumita Panigrahi
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Date:16/07/2020

Place: Bhubaneswar (***Sumita Panigrahi***)

CONTENTS

LIST OF ABBREVIATIONS

%	Percentage
TB	Tuberculosis
PTB	Pulmonary tuberculosis
EPTB	Extra pulmonary tuberculosis
MTB	Mycobacterium tuberculosis
RIF	Rifampicin
CBNAAT	Cartridge-based nucleic acid amplification test
RNTCP	Revised National Tuberculosis Control Program
DOTS	Directly observed treatment, short course
HIV	Human immune virus
NSP	National Strategic Plan
BCG	Bacille Calmette Guerin
PCR	Polymerase chain reaction
WHO	World Health Organization
SPC	Sample processing unit
PCC	Probe check control
DNA	Deoxy ribonucleic acid
CT	Cycle threshold
DSTB	Drug sensitive tuberculosis
rpoβ	β-subunit of RNA Polymerase
PHC_S	Public healthcare services
FM	Fluorescence microscopy
TTP	Time to positivity

ABSTRACT

TB is an airborne infectious disease however it is curable and preventable. According to WHO, Directly Observed Treatment, Short Course(DOTS) is the most effective way to cure it to stop the transmission of TB in communities. It helps patients to finish the course of TB as quickly as possible without unnecessary gaps or treatment failure. Healthcare workers observe the patients timely in the programme. But still adherence to DOTS is a challenge in many countries due to various factors such as improper follow up, treatment in private sector, alcoholism, lack of awareness. As in India 56% of the TB patients visit the private health sector which is very unstructured and unregulated and there is no mechanism to follow up the patient till they are declared cured. Failure and default in treatment leads to relapse of the disease which leads to multidrug resistant (MDR) TB. So, National TB Elimination Program (NTEP) sensitized the private sector to notify the patient to the program and refer the patient for Cartridge Based Nucleic Acid Amplification Test (CBNAAT) testing under Universal DST (UDST) for all the notified TB patients. The aim of the current study is to do a questionnaire-based data collection and follow up the patients referred by different private health facilities of Bhubaneswar to know about their current treatment facility, health status and its outcome. On account of that, samples referred by the private healthcare facilities to National Reference Laboratory were tested by CBNAAT. The TB positive patient's details were collected from the lab register to call the patient with a set of questionnaires to know their DOTS adherence status and its determinants.

CHAPTER -1

INTRODUCTION

CHAPTER: 1 INTRODUCTION

Tuberculosis(TB) is an air borne infectious disease caused by *Mycobacterium tuberculosis*, which spread from person to person through air droplet, for example by coughing, sneezing. It is one of the top 10 causes of death worldwide and as per World TB report of 2019 an estimated 10 million people infected with TB and a total of 1.5 million people died (including 2,51,000 people with HIV) in 2018 worldwide. The TB incidence of India is 2.7 million in 2018 of which 25 % was from the private sector. TB is the highest killer among the infectious disease in India. Majority of the TB burden is among the working age group. The 89% of TB cases come from the age group of 15-69 years. The morbidity and mortality of TB among women and children is larger than often realized. About 2/3 of the TB cases are Males.

In order to control TB, a highly specific and sensitive rapid diagnostic test is prerequisite for early diagnosis and effective treatment of TB patients there by breaking the transmission chain. In December 2010, WHO recommended use of CBNAAT for detection of pulmonary and extra-pulmonary (EP) TB along with rifampicin resistance status. In India more than half of all TB patients seek care in the private sector. Under the goal "Universal access to TB care and treatment for all" of RNTCP's National Strategic Plan (NSP) 2012-17, upfront CBNAAT test was offered to patients from the private sector.

The WHO recommended strategy to combat TB, directly observed treatment, short-course (DOTS) that can cure over 85% of patients with TB. DOTS is the most effective strategy available for controlling the TB epidemic today. The strategy must be adapted to fit specific country situations. Since the introduction of the strategy, great strides have been made in spreading the message to governments, health care workers and the public about the importance of implementing DOTS. This cost-effective strategy was developed from the collective best practices, clinical trials and programmatic operations of TB control over the past two decades. It differs from other control approaches, and its role is challenging in changing health care system.

An important feature of DOTS is the basic management unit, that covers a huge population and has the staff and resources to diagnose, initiate the treatment, record and report patient treatment progress and manage supplies. Recording and reporting system is used by

healthcare workers to systematically monitor patient progress and TB programme performance.

TB is curable and preventable but there are various factors play a crucial role in patient adherence to DOTS in the epidemic areas. Globally, TB is falling at about 2% per year. An estimated 58 million lives were saved through TB diagnosis and treatment between 2000 and 2018. Majority of the factors include poverty, addiction, loss of daily wagers, lack of awareness, family problem, lack of proper counselling by health workers and relatives.

In this study we have showed the involvement of private sector in availing the TB diagnosis facility of a government set up and contributing to TB notification. The primary objective of the study was to know the determinants of DOTS adherence by the CBNAAT positive TB patients referred by private health facilities of Bhubaneswar.

Objective:

1. To assess the involvement of private sector in availing the TB diagnosis facility and contributing to TB notification.
2. To identify determinants of DOTS adherence by the CBNAAT positive TB patients referred by private health facilities of Bhubaneswar.

Plan of work:

1. Study population: Patients referred from private health facilities for CBNAAT testing.
2. Specimen processing: Pulmonary and extra pulmonary specimens were processed for CBNAAT as described in RNTCP Technical and Operational Guideline-2016 and WHO Xpert MTB/RIF Implementation Manual.
3. Data Collection: Data collection will be done from the respective lab register Missing data and the data/determinants for treatment adherence will be collected from patients by telephonic conversation and using a pretested questionnaire. Verbal consent will be taken for using the data for research purpose.
4. Data Analysis: Validation of data, Calculation of simple proportions of selected demographic, behavioral, and clinical characteristics among cases with TB as the underlying cause.

Proposed Outcome:

Determinants for DOTS adherence for CBNAAT positive TB patients referred by private health facilities of Bhubaneswar will be assessed.

CHAPTER -2

REVIEW OF LITERATURE

CHAPTER: 2 REVIEW OF LITERATURE

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* which is transmitted by air droplet. The bacteria discovered by Robert Koch on 24th March 1882, for which he was awarded with noble prize in Medicine and Physiology in 1905. The term "Tuberculosis" was coined by Johann Schonlein in 1834. Albert Calmette and Camille Guerin achieved the first Guerin success in immunization against TB by using attenuated bovine strain tuberculosis which was named Bacille Calmette Guerin (BCG). On humans, BCG vaccine was first used in 1921 in France. TB can affect any organ of the body. So, based on the site of infection there are two types of TB, pulmonary TB (PTB) that affect the lungs and extra-pulmonary TB tuberculosis (EP-TB) that affect other parts of the body. Pulmonary TB is the most common TB with symptoms like chronic cough with blood containing sputum, fever, night sweats, loss of appetite, unexpected weight loss i.e. 5% weight loss in 3 months, fatigue and significant nail clubbing may occur. The diagnosis of PTB is easier than the EP-TB because the symptoms of EP-TB is not specific, collection of EP-TB samples are difficult in peripheral health facilities and the samples are paucibacillary in nature.

CBNAAT is a point of care (POC) testing with detection limit of 131cfu/ml in comparison to 5000-10000 cfu/ml by smear microscopy. The CBNAAT for use with the Cepheid GeneXpert®Dx system is a real-time PCR *in-vitro* diagnostic test for: (1) the detection of MTB DNA in samples (2) the detection of rifampicin resistance associated mutations of the *rpoB* gene within two hours. It incorporates and automates sample processing, nucleic acid amplification, and detection of the target sequences. The system requires the usage of single-use disposable XpertMTB/RIF cartridges that hold the reagents of PCR and host the PCR process entirely. A sample processing control (SPC) is also present in the cartridge to control the adequate processing of target bacteria and the presence of inhibitor(s) in the PCR reaction is monitored. The Probe Check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers present in the XpertMTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes differentiate between the conserved wild-type sequence and mutations in the core region that are linked with rifampicin resistance **(Lawn and Nicol, 2012).**

WHO recommended use of CBNAAT in December 2010, for detection of PTB and EP-TB along with rifampicin resistance. As per the policy testimonial by WHO in 2013, under Revised National Tuberculosis Control Program (RNTCP), to diagnose pulmonary TB, paediatric TB, EPTB and TB, CBNAAT is being used in preference to conventional microscopy and culture as the initial diagnostic test in high risk population such as HIV-positive cases. Given the lack of data on utility, CBNAAT is not recommended to examine the specimens like stool, urine or blood. **(WHO Xpert MTB/RIF implementation manual, 2014).**

Directly observed treatment, short-course (DOTS, also known as TB-DOTS) is the name given to the TB control strategy recommended by the World Health Organization. According to WHO, "The most cost-effective way to stop the spread of TB in communities with a high incidence is by curing it. The best curative method for TB is known as DOTS". The technical strategy for DOTS was developed by Karel Styblo of the International Union Against TB & Lung Disease in the 1970s and 80s. In 1994, WHO adopted the DOTS for the worldwide control of TB. All countries with a TB problem were to provide standardized short course drug treatment to, at least, all sputum smear positive TB patients. Until 2006 DOTS was to be the internationally recommended approach to global TB control. In 1996 the WHO claimed that "where the health system is working even moderately well, the DOTS strategy is extraordinarily effective achieving cure rates over 90%." In 1999 WHO and their partners launched DOTS-Plus for the treatment of MDR TB. (https://tbfacts.org/dots-tb/)

Queiroz et al.;(2012) study and analyse the limitations and strength of DOTS from the patients and health care providers in SP, Brazil. 4 patients and 11 health care providers were interviewed. The strengths were: bonds between healthcare providers and patients and the incentives were introduced promoting treatment adherence. Limitations included: patient supervision and DOTS healthcare provider's involvement is restricted. They found that the need to go beyond the medical supervision should be acknowledged by the healthcare providers.

Ershova et al.;(2014) conducted a study to evaluate the adherence to standardise TB treatment, provision of DOTS and analyse its impact on treatment outcome in 3 provinces of South Africa. They collected data from 1339 patients and found that 749 patients got proper

treatment and patients who received incomplete DOTS treatment or had a history of defaulting treatment had an increased risk of poor outcomes.

Rondangs et al.;(2014) conducted semi structured interviews with non-adherent patients in Central Java, Indonesia. 3 non-adherent reasons they found were knowledge about TB, their treatment and choosing a health care facility. They found that there was lack of knowledge among the people.

Sheotani et al.;(2014) studied socio cultural influences on adherence to tuberculosis treatment in rural parts of India. They found poor adherence to treatment by DOTS is a major problem. They interviewed the patients and health care providers and found mismatch between the Directly observed treatment short course(DOTS) and the social norms.

Dewan et al.;(2015) studied and compared the efficacy of sputum microscopy and CBNAAT for diagnosing pulmonary TB. Analytical cross study was made of the sputum samples and CBNAAT was done. Out of many patients, 11 were found positive by microscopy method and 40 by CBNAAT. CBNAAT helps in increased case detection in lesser time in pulmonary TB patients.

Das et al.;(2015) conducted study to assess the adherence rate among pulmonary tuberculosis patients and to study the factors affecting adherence to DOTS programme in West Tripura that included 220 patients from various districts. They found good percentile of adherence rate among the people despite of poor connectivity and other problems.

Lande et al.;(2016) highlighted the recent initiatives under RNTCP, in which they referred TB patients for HIV counselling and testing. They expanded recording of HIV status with TB programme.

Ndwiga et al.;(2016) conducted study to know the factors influencing knowledge on completion of treatment among TB patients under directly observed treatment strategy in health facilities in Embu, Kenya. Cross-sectional study was conducted among the population and concluded that TB programmes should come up with better ways to educate patients on the importance of supervision and treatment completion during the treatment of TB.

Mandal et al.;(2017) studied that in 1992, Government of India follow the internationally recommended DOTS strategy based on five principles, political and administrative commitment, good-quality diagnosis, uninterrupted supply of quality drugs, DOT, and systematic monitoring and accountability. Following a pilot programme from 1993 to 1996, the RNTCP was officially launched in 1997 and amplified in a phased manner to all the districts in the country by 2006.

Mukherjee et al.;(2017) evaluated the CBNAAT in diagnosis of pulmonary TB patients. They studied that CBNAAT significantly adds to the diagnostic yield of PTB in comparison. It has additional advantage of identifying rifampicin resistance with high sensitivity and specificity.

Kandi et al.;(2017) conducted a study at Government General and Chest Hospital, Hyderabad, India from 2014 to 2016. The studied all the pulmonary and extra pulmonary presumptive TB cases. They found CBNAAT to be more specific and sensitive in diagnosis of TB.

Sahille et al.;(2018) interviewed 10 TB patients attending DOTS in two public health centres in Ethiopia. Interviews were tape recorded and analysis was done to know the experiences and perceptions of TB treatment adherence.

Muller et al.;(2018) performed a systematic review and meta-analysis of 22 clinical trials to ascertain whether providing DOTS and other incentives improved or not, where they found increase in cure rates among the patients.

Ruru et al.;(2018) conducted a case control study to identify factors like access to healthcare, TB knowledge and treatment experience associated with non-adherence during TB treatment among patients treated at public healthcare services(PHCs) in Jayapura, Indonesia.

Salinitia et al.;(2018) studied and compared CBNAAT with that of fluorescence microscopy(FM) in the diagnosis of pulmonary tuberculosis. A cross-sectional study was done among 200 patients and 3 sputum samples and 2 slide smears were prepared from each patient sample and they found 17% samples were found positive by FM and 29% by CBNAAT. So, they concluded CBNAAT is a better method of detecting PTB as compared to FM.

Bhattacharya et al.;(2018) studied the factors contributing to non-adherence among defaulter TB patients and to know the barriers of DOTS in the locality of Burdwan, Bengal. Interviews were taken by 14 defaulters and health care providers of the centres and the staff members and they found various reasons resulting in default those were found to be addiction, loss of daily wagers, lack of awareness, family problem, poverty, lack of proper counselling by friends.

Prakash et al.;(2018) studied and estimated the diagnostic accuracy and additional yield of Xpert MTB/RIF in various specimens and association between the CT value (low, medium, high) and culture time to positivity(TTP). 1000 samples of pulmonary and extra-pulmonary patients were collected from patients of a tertiary care hospital and sensitivity and

specificity of CBNAAT and the relation between CT value and TTP is studied. They found different percentile of sensitivity of CBNAAT for different specimens. They found CBNAAT to be a robust and accurate test for PTB and EPTB and the relation between CT and TTP value indicates the bacterial load present.

Tatti et al.;(2019) assessed the quality of DOTS adherence at a national TB hospital among MDR-TB and DS-TB patients using a questionnaire and found that the adherence counselling is sub-optimal.

Chakrabartty et al.;(2019) explored the stigma perceived by the persons with TB and its influence on the adherence to DOTS treatment. An epidemiological study was conducted using a questionnaire among 145 DOTS defaulters from random districts in West Bengal.

RashmiAgarwalla et al.;(2020) conducted study among 50 private practitioners of Delhi over a period of 3 months. Then data collection and interviews were done and found out that 42% were adherent and 58% were non-adherent among the list of 50 practitioners. They evaluated the current situation of diagnosis and treatment of TB in Delhi.

CHAPTER -3

MATERIALS AND METHODS

CHAPTER: 3 MATERIALS AND METHODS

A. Study Population:

CBNAAT positive TB patients referred by different private healthcare facilities of Bhubaneswar for CBNAAT testing at NRL RMRC Bhubaneswar.

B. Sample Collection:

Pulmonary and extra pulmonary sample of the patients has been received in a sterile vial and tested by CBNAAT.

C. Specimen processing:

Pulmonary sample includes Sputum and BAL (Brancho Alveolar Lavage) whereas extra pulmonary sample includes pus, soft tissues (for e.g. Lymph Node Biopsy, Punch Biopsy, Endometrium tissue) and other body fluids such as, Gastric Lavage, Fine Needle Aspiration Sample, CSF (Fluid from spinal cord) and Pleural Fluid.

1. Procedures

1.1. General considerations

Important points about specimen processing procedures

- Ensure that the GeneXpert MTB/RIF cartridge are labelled correctly and clearly.
- Tissues and lymph node must be processed within a biological safety cabinet, given the risk of producing aerosols while grinding and homogenizing samples.
- CSF samples are paucibacillary and can be processed using the same precautions as those used for sputum EXCEPT when they are concentrated by centrifugation.
- It is important to use Safe Working Practices to avoid contamination by bacteria other than tubercle bacilli and specially to avoid cross-contamination with tubercle bacilli from other specimens.
- Exposure time to decontamination reagents must be strictly controlled for samples requiring decontamination.
- Decontaminate samples by NaOH-NALC method depending on the quality of sample.

- Do not open the Xpert MTB/RIF cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped or shaken after you have added the treated sample.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use a cartridge that has a damaged reaction tube.
- Don't use cartridges after expiry date.
- Each single-use Xpert MTB/RIF cartridge is used to process one test. Do not reuse spent cartridges.
- Write the sample ID on the sides of the cartridge or affix ID label.
- Do not put the label on the lid of the cartridge or obstruct the existing 2D barcode on the cartridge.
- Don't touch the barcode while loading the cartridge in to the module.

1.2. Specimen Processing

Process only as many samples at one time as there are modules available to run the test on the GeneXpertDx System.

1.2.1. Expectorated Sputum Samples

- Label each Xpert MTB/RIF cartridge with the sample ID.
- Leave specimen in leak-proof sputum collection container.
- For each of the samples; unscrew lid of sputum collection container; add Sample Reagent 2:1 (v/v) to sample, replace the lid, and shake vigorously 10 - 20 times. Note: One back-and-forth movement is a single shake.
- Incubate at room temperature. After 10 min. of incubation, shake the specimen vigorously 10-20 times.
- Specimen should now stand for 5 minutes.
- Samples should be liquefied perfectly with no visible clumps of sputum.
- Increase the incubation time for 10-15 mins if sample is not completely liquefied.

- Transfer 2 ml of processed sample to cartridge.

1.2.2. Processing of Pus Sample

Note: Generally, this sample is viscous / thick and it may have blood

Viscous Pus sample without blood stains will be processed as follows

- Treat 1 ml of pus with 2 ml of sample reagent (1:2 Ratio, Pus:SR)
- Shake vigorously 10 to 20 times or vortex for at least 10 seconds. Note: One back-and-forth movement is a single shake.
- Incubate at room temperature. After 10 min. of incubation, shake the specimen vigorously 10-20 times.
- Specimen should now stand for 5 minutes.
- Samples should be liquefied perfectly with no visible clumps of sputum.
- Increase the incubation time by 10-15 mins if sample is not liquified properly.
- Label each Xpert MTB/RIF cartridge with the sample ID.
- Transfer 2 ml of processed sample to cartridge.

1.2.3. Processing of Soft tissues (for e.g.- Punch Biopsy, Lymph Node Biopsy, Endometrium Tissue)

Note: The tissue sample should be transported in sterile container having Normal Saline or Sterile Water. (Avoid processing the tissues dipped in formalin)

All tissue samples, without blood stains will be processed as follows

- First wash out the normal saline or water used in transport container by taking out tissue in sterile petri dish, rinse it with sterile water, remove the washings
- Use 1 cm x 1 cm of clean piece of tissue for chopping using sterile surgical blade to very tiny pieces
- Add little bit of Sample reagent to it, mix the same & then transfer the entire content to sterile conical bottom screw capped tube
- Add sample reagent to keep 1:2 Ratio (Tissue: SR)
- Shake vigorously 10 to 20 times or vortex for at least 10 seconds. Note: One back-

and-forth movement is a single shake.

- Incubate at room temperature. After 10 min. of incubation, shake the specimen vigorously 10-20 times.
- Specimen should now stand for 5 minutes.
- Samples should be liquefied perfectly with no visible clumps of sputum.
- Label each Xpert MTB/RIF cartridge with the sample ID.
- Transfer 2 ml of processed sample to cartridge.

1.2.4. Processing of Other Body Fluids such as- BAL (Broncho Alveolar Lavage), Gastric Lavage, Fine Needle Aspiration Sample, Synovial Fluid, Pleural Fluid

All above sample type without any blood will be processed by following method

- Add sample reagent to keep 1:2 Ratio (Sample:SR)
- Shake vigorously 10 to 20 times or vortex for at least 10 seconds. Note: One back-and-forth movement is a single shake.
- Incubate at room temperature. After 10 min. of incubation, shake the specimen vigorously 10-20 times.
- Specimen should now stand for 5 minutes.
- Samples should be liquefied perfectly with no visible clumps of sputum.
- Label each Xpert MTB/RIF cartridge with the sample ID.
- Transfer 2 ml of processed sample to cartridge.

1.2.5. CSF (Fluid from Spinal Cord)

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 all of the 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.
- NOTE: Concentrated CSF should be decanted within a biological safety cabinet
- Re-suspend 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 ID.
- 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 (SR) 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

- Re-suspend 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.

If blood stains are seen in CSF sample

- Process the sample by NALC NaOH Precipitation (Digestion, Decontamination) method
- Treat 1 ml of precipitate/ sediments with 2 ml of sample reagent & follow 15 minutes mixing & incubation protocol (If less sediment is obtained then use at least

0.5 ml sediment / precipitate & mix it with 2.5 ml sample reagent and then follow 15 minutes mixing & incubation protocols)

1.3. Preparation of Cartridges

Important: 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. Do not process the sample further if there is insufficient volume.
- Open the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge. See Fig. 1, below. Dispense slowly to minimize the risk of aerosol formation.
- Close the cartridge lid. Make sure the lid snaps firmly into place. Remaining liquefied sample may be kept for up to 12 hours at 2 – 8 ºC should repeat testing be required.
- During transferring the sample in to the cartridge avoid the solid particles and air bubble formation.
- Wipe the cartridge outer surface and the top portion using disinfectant.

Important: Be sure to load the cartridge into the GeneXpertDx instrument and start the test within 30 minutes of preparing the cartridge.

1.4. Starting the Test

Important: Before you start the test, ensure that the system is equipped with the GX 4.6a software, and the Xpert MTB/RIF assay is imported into the software.

- This section lists the basic steps of running the test. For detailed instructions, see the GeneXpert System Operator Manual.
- Turn on the computer, and then turn on the GeneXpert instrument.
- On the Windows® desktop, double-click the GeneXpert shortcut icon.
- Log on to the GeneXpert System software using your user name and password.
- In the GeneXpert System window, click Create Test. The Scan Cartridge Barcode dialog box appears.

- Scan the barcode on the GeneXpert MTB/RIF cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
- In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the "View Results" window and all the reports.
- Click Start Test. In the dialog box that appears, type your password.
- Open the instrument module door with the blinking green light and load the cartridge.
- Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
- Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.
- Dispose of used cartridges in the appropriate specimen waste containers according to standard practices (SOP Waste Management).
- Viewing Results
- For detailed instructions on how to view and print the results, (Cepheid GeneXpertDx System Operator Manual).

CHAPTER -4

RESULTS AND DISCUSSION

- MTB Detected/RIF Resistance Not Detected- TB positive with Rif sensitive
- MTB Detected/RIF Resistance Detected- TB positive with Rif resistance
- MTB Not Detected- TB negative

Data collection for DOTS adherence study:

Demographic data of all the TB patients is collected from the lab register between the study period. A set of questionnaires has been developed to collect data from the patient regarding their adherence to DOTS and their current treatment health facility and outcome by telephonic conversation. The questions are developed to know the determinants for treatment adherence and how they overcome any adverse reaction during the treatment. Verbal consent will be taken for using the data for research purpose. The data will be analyzed by SPSS statistics software package.

Future outlook

Data will be collected from the register and verbal consent will be taken for study and research purpose.

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