Evaluation of the Antibiotic Resistance Pattern of Mycobacterium Tuberculosis Isolated from Tb Patients of Kamrup District, Assam, India

Bipul Chandra Deka

Research scholar, Program of Biotechnology, Assam down town University, Sankar Madhab Path, Gandhi Nagar Panikhaiti, Guwahati, Assam 781026.

Dr Devabrata Saikia*

Assistant Professor, Program of Biotechnology, Assam down town University, Sankar Madhab Path, Gandhi Nagar Panikhaiti, Guwahati, Assam 781026.
Email: devabrata.saikia@adtu.in

*Corresponding author:

devabrata.saikia@adtu.in

Abstract

Background: Tuberculosis (TB) is an infectious disease caused by the bacterium Mycobacterium tuberculosis. It primarily affects the lungs, but can also affect other parts of the body. Symptoms include coughing, chest pain, fever, and weight loss. TB is spread through the air when an infected person coughs, sneezes, or talks. It is treated with a combination of antibiotics for a period of several months. Tuberculosis is one of the most serious health issues of the world which is associated with antitubercular drug resistance. Methods: Sputum was collected from presumptive TB patients, those who attended OPD of nine BPHC of Kamrup district, Assam and determined the drug susceptibility of Mycobacterium tuberculosis isolated from sputum positive patients with freshly diagnosed pulmonary TB by using ZN microscope. and DST (culture independent). Results: Out of 199, sputum of 85 number of TB patients were subjected to GeneXpert and LPA. It was observed that, 20% and 6% samples were resistant to the antibiotics rifampicin and Isoniazid respectively. Moreover, multidrug resistance pattern was also observed. 21.17% were found to be both rifampicin and isoniazid resistant, 1.17% Rifampicin and Isoniazid and levofloxacin resistant, 1.17% were rifampicin, levofloxacin and moxifloxacin resistant, again another 1.17% Rifampicin and Levofloxacin resistant. Conclusion: The study highlights the burden of antitubercular drug resistance and established that need of DST in the affected area.

1. Introduction:

Tuberculosis is an infectious and common air born disease caused by *Mycobacterium tuberculosis.* Tuberculosis has a very ancient history of existence as it was discovered in fossils dated to 17,000 years before the present (BP) [1]. Tuberculosis was once a common cause of death in the United States and Europe, which led to the death of one out of seven people during the late 1880s. India is also not lagging behind in this aspect. In 1911, the first All India Sanitary Conference was held in Bombay. In

that conference it was disclosed that in some big cities of India like Calcutta, and Bombay the mortality rate due to TB is higher than Glasgow, Birmingham or Manchester in the UK [2]. This bacterium was discovered by Robert Koch in 1882 and 24th march is celebrated as world TB Day-across the world.

As Tuberculosis is an air born disease, the infection rate is very high and an infected person may spread the infection to ten to fifteen normal people if not quarantined or treated on time [3]. The symptoms of

tuberculosis include prolonged cough and fever generally more than two weeks.-Tuberculosis is the thirteenth leading cause of death worldwide and second leading infectious killer after COVID-19 in 2021.In 2021 1.6 million people of the entire globe died from tuberculosis [4]. The Covid-19 pandemic has worsened the TB epidemic globally and paralysed the National TB programmes worldwide due to the fact that both of the diseases are airborne in nature and comorbidities are almost identical. COVID-19 and tuberculosis are two diseases which have significant threat to health system. Some study reports showed that COVID-19 positive patient along with tuberculosis resulted more death that only COVID -19 Positive patients. So, it was recommended that routine screening of tuberculosis may be advisable among COVID patients or presumptive COVID -19 patients [5].

Various tools are used to diagnose TB in humans. Despite the introduction of numerous new tools and techniques, microscopy remains the gold standard tool for the detection of TB. [6]. The sputum microscopy is highly specific in area with a high prevalence of tuberculosis. But as per performance concern sputum microscopy has some significant limitation. With sputum microscopy the sensitivity is compromised when the bacterial load is less than 10000 bacilli/ML sputum sample but to monitor the progress of infection and for giving final result of cure sputum microscopy is being used [7].

As a part of the development of modern technique, automated real time sputum processing Xpert MTB/RIF assay are developed for the detection of tuberculosis. But due to relatively high operating cost it is being used within limitation [8]. The Xpert MTB/RIF was found to be highly sensitive (98%) and specific (98%) in detecting tuberculosis and to know the rifampicin resistance status within 2 hours. It is very easy to perform. The molecular based methods detect nucleic acid materials of *Mycobacterium tuberculosis*. Gene xpert and LPA methods are both capable of detecting mutation causing resistance against rifampicin. In addition, LPA can detect mutations related to isoniazid.

India bears the highest TB burden (27%) followed by China (9%) and Indonesia (8%) [9]. Assam is a small state of India inhabited with different tribes and communities. In Assam, the total number of notified TB cases were 37641 in 2021. Kamrup district is one of the largest districts of Assam having population of 15.18 lakhs and area is 3105 square kilometres with a variable demography [10].

The aim of study is to detect tuberculosis from sputum samples, microscopy, GeneXpert RIF assay, LPA are the widely used methods. As per sensitivity and specificity concern which process is best for detection of tuberculosis. LPA is a robust technique for detection of *Mycobacterium tuberculosis*. Sometimes *Mycobacterium tuberculosis* may not be detected in sputum samples by LPA, although they are smear positive by smear microscopy. This may be due to presence of non-tubercular mycobacterium (NTM) [11].

Sputum microscopy the sensitivity is compromised when the bacterial load is less than 10000 bacilli/ML sputum sample but to monitor the progress of infection and for giving final result of cure sputum microscopy is being used [12]

2.   Aim of Study:

For detection of tuberculosis from sputum samples, microscopy, GeneXpert RIF assay, LPA are the well accepted methods. By using these methods detection of antibiotic resistant strains from TB patients and discussion on this retrospective analysis was attempted to understand the importance of LPA for early detection of antibiotic resistant strains

3.   Materials and Method:

3.1: Sample size, study design:

Presumptive TB patients from 9 block public health centre of Kamrup district were included Suwalkuchi BPHC, Bishnuram Medhi FRU, Hajo, Boko BPHC, Chayygaon Block public health center, North Guwahati BPHC, Rangiya BPHC, Bihdia BPHC, Nagarbera BPHC, Azara BPHC, where sputum samples were collected from January 2022 to September 2022 from all age group individuals who were clinically diagnosed as presumptive TB.

3.2: Inclusion and exclusion criteria:

3.2.1. Inclusion criteria:

Journal of Coastal Life Medicine

Patients of all ages and gender having symptoms of pulmonary tuberculosis were included in the study.

3.2.2. Exclusion criteria:

Patients having extra pulmonary tuberculosis were excluded in the study.

3.3: Ethical approval:

From the Joint Director of Health Services, Kamrup, approval for the work was taken vide reference number DHS/K/NTEP/2020/195/ dated 06/06/2020. The confidentiality and privacy were maintained.

3.4.: Specimen collection:

From the presumptive TB patients, one sputum samples were collected on the spot and another were collected within a gap of one hour from the first one in sterile falcon tube. Sputum with blood and less than 2 ML were discarded in the study.

3.5: Microscopy:

Using a sterile small bamboo stick, a drop of sputum was placed on a clean glass slide and heated and fixed and Z N staining (Ziehl- Neelson method) process was done as per NTEP guideline.

3.6: GeneXpert MTB -RIF assay:

Cartridge based nucleic acid amplification test (CBNAAT) is conducted in GeneXpert MTB/RIF machine for simultaneous rapid tuberculosis diagnosis and rapid antibiotic sensitivity test. The test was done as per instruction from manufacturer. The collected sputum samples were recorded and with a help of a pipette added 2 ML of sodium hydroxide and isopropanol containing sample reagent (SR) to 1 ML of sputum samples and incubated in room temperature for 15 minutes. After that 2 ML of the treated sample solution was transferred to a cartridge and loaded in GeneXpert machine. After 2 hrs result was available for study.

3.4.3: Line probe assay:

LPA based on strip technology was used for detection of Rifampicin and Isoniazid resistance due to mutation of *rpoB* and *inhA* and *catG* genes. DNA extraction, PCR amplification, and hybridization are the process that were done as per manufacturer's protocol (Hain Life Sciences) [13].
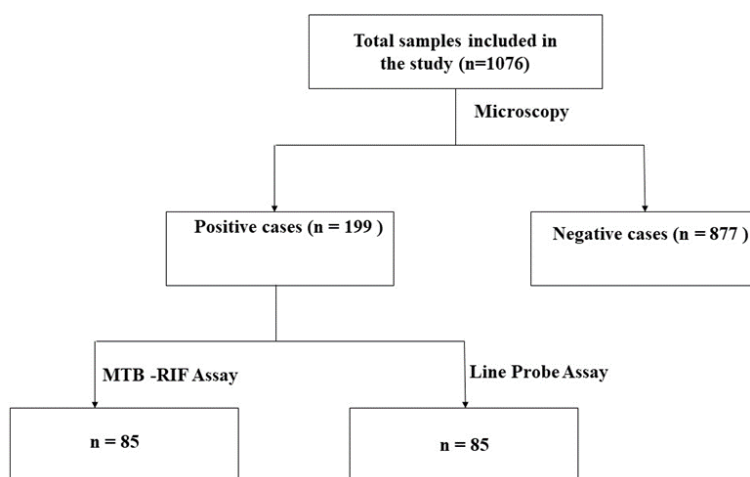
4. Result and Discussion:

Figure 1: Algorithm for the diagnosis of Tuberculosis

From nine BPHC of Kamrup district, sputum samples were collected from 1076 presumptive patients. Out of 1076 only 199 (18.49%) people were found positive by sputum microscopy where 153 (76.8%) patients were male and 46(23.1%) were female as shown in the table 1. In the age group of 16-30, maximum number of TB patient were detected.

Journal of Coastal Life Medicine

Table 1: Microscopic analysis of the patients based on age and sex

Age group	Male	Female	Total (%)
<15	4	1	5 (2.51)
16-30	51	19	70 (35.1)
31-45	50	9	59 (29.64)
46-60	30	14	44 (22.11)
>60	18	3	21 (10.55)
Grand total	153 (79.89)	46 (23.11)	199

Out of 199, sputum of 85 number of TB patients were subjected to GeneXpert and LPA. Out of 85 samples 58 (68.23%). were from male and 27(31.76%) were from female patients. and 17 (20%) were found rifampicin resistance, 6 (7.05%,) were found Isoniazid resistance, 18(21.17%) were found both rifampicin and isoniazid resistance, 1 (1.17) were found Rifampicin and Isoniazid and levofloxacin resistance, another 1 (1.17) were found rifampicin and levofloxacin and moxifloxacin resistance, again another 1 (1.17) were found Rifampicin and Levofloxacin respectively and lastly 41 (48.2%) were found sensitive to all anti tubercular drug resistance as shown in the table 2.

Table 2: Antibiotic resistance pattern of different isolates

Drugs	Male	Female	Total (%)
Rif	12	5	17 (20%)
Iso	4	2	6 (7.05%,)
Rif & Iso	10	8	18(21.17%)
Rif& Iso& Levo	1	0	1 (1.17)
Rif& Levo& Moxi	1	0	1 (1.17)
Rif& Levo	1	0	1 (1.17)
All anti Tb drug sensitive	29	12	41 (48.2%)
Total	58	27	85

Rifampicin -(Rif), Isoniazid-Iso, Levofloxacin-Levo, Moxifloxacin- Moxi,

5. Discussion:

Tuberculosis is infectious air born disease. It may attack any people of any age group. But it has been established in this study that tubercular infection is more in the age group of (16-30) and in compared to female population, number of male patients are more. But the actual causes are not known. The actual cause is not known but one report established that male can expelled out good quality of sputum for smear examination and female is unable to do so. Another study showed that female population is less than male in case of detection of tuberculosis, because of social stigma, cultural barrier in female in case of sputum collection [15. 16] Word Health organization has also encouraged to emphasize on research involving TB screening for woman.

Although TB is curable but drug resistance trend among tubercular patients is not good sign for health system. 20% TB patients shows resistance to Rifampicin and 21% TB patients shows resistance to both Rifampicin and Isoniazid. These statistics is totally unhealthy for our society. As more than 50% TB patients shown resistance more than one anti tubercular drug means either our health set up is failure to treat the TB patients properly or treatment may be relapse. It has also been reported that in some cases TB patients are taking treatment very secretly due to social stigma. 50 % of TB patients got scads to disclose their Tb disease among their family and friends. Age group of below 30 years faced high stigma at their workplace. So successful treatment outcome is less. In our study it may happened. So need of further study is required in this regards. [17]

Journal of Coastal Life Medicine

6. Conclusion:

More number of drug resistant TB cases occurred. Route cause is not clear. May be social stigmatization or lack of knowledge about the TB disease. Solid action plan is required. Health department and social activist, NGO's intervention may be helpful.

Reference:

1. Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. Journal of preventive medicine and hygiene. 2017;58(1): E9.

2. Baheti S, Dighe D, Kumbhar A, Prasad A. Exploration of Ayurveda Potential In Tuberculosis: Current Scenario And Future Prospect. International Journal of Ayurveda and Pharma Research. 2020; 32:19-32.

3. Chin DP, Crane CM, Diul MY, Sun SJ, Agraz R, Taylor S, Desmond E, Wise F. Spread of Mycobacterium tuberculosis in a community implementing recommended elements of tuberculosis control. Jama. 2000; 283(22):2968-74

4. Mehta B, Chawla S, Kumar Dharma V, Jindal H, Bhatt B. Adult immunization: the need to address. Human vaccines & immunotherapeutics. 2014;10(2):306-9

5. Song WM, Zhao JY, Zhang QY, Liu SQ, Zhu XH, An QQ, Xu TT, Li SJ, Liu JY, Tao NN, Liu Y. COVID-19 and tuberculosis coinfection: an overview of case reports/case series and meta-analysis. Frontiers in medicine. 2021; 8:657006

6. Nyendak MR, Lewinsohn DA, Lewinsohn DM. New diagnostic methods for tuberculosis. Current opinion in infectious diseases. 2009;22(2):174-82.

7. Desikan P. Sputum smear microscopy in tuberculosis: is it still relevant? The Indian journal of medical research. 2013;137(3):442.

8. Deka BC, Saikia D, pratim Kashyap M. Diagnosis of Tuberculosis. European Journal of Molecular & Clinical Medicine.;9(07):2022.

9. World Health Organization. Global tuberculosis report 2019. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO

10. Deka BC, Saikia D, Kashyap MP, Sarma JB, jyoti Kakati N. Screening for Pulmonary Tuberculosis among Patients with Cough Attending Different Block Public Health Centre of North Bank of Kamrup District of Assam, India: Does Location, Gender and Duration of Cough Impact on Screening?.

11. Prabha Desikan et al: Sputum smear microscopy in tuberculosis: Is it still relevant? Indian J Med Res 137, March 2013, pp442-444. prabhadesikan@yahoo.com

12. Kakati NJ. A study of MDR TB among sputum positive pulmonary TB patient (In a tertiary care centre) with special emphasis on patient characteristics. PhD thesis, 2017 Srimanta Sankaradeva University of Health Sciences Guwahati Assam.

13. Desikan P, Panwalkar N, Mirza SB, Chaturvedi A, Ansari K, Varathe R, Chourey M, Kumar P, Pandey M. Line probe assay for detection of Mycobacterium tuberculosis complex: An experience from Central India. The Indian journal of medical research. 2017 Jan;145(1):70.

14. Tukvadze N, Bablishvili N, Apsindzelashvili R, Blumberg HM, Kempker RR. Performance of the MTBDRsl assay in Georgia. The International journal of tuberculosis and lung disease. 2014 Feb 1;18(2):233-9

15. Rao S. Tuberculosis and patient gender: An analysis and its implications in tuberculosis control. Lung India: Official Organ of Indian Chest Society. 2009 Apr;26(2):46.

16. Begum V, De Colombani P, Das Gupta S, Salim Md A H, Hussain H, Pietroni M, Rahman S, Pahan D, Borgdorff M W. Tuberculosis and patient gender in Bangladesh: sex differences in diagnosis and

Journal of Coastal Life Medicine

treatment outcome. The International Journal of Tuberculosis and Lung Disease. 2001 Jul 1;5(7):604-10.

17. Dashti S, Dabaghi P, Tofangchiha S. The effectiveness of training program based on protective motivation theory on improving nutritional behaviors and physical activity in military patients with type 2 diabetes mellitus. Journal of family medicine and primary care. 2020 Jul;9(7):3328