ORIGINAL RESEARCH PAPER
Volume-9 | Issue-2 | February-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PATTERN OF RIFAMPICIN RESISTANCE (RIF) IN CBNAT POSITIVE CASES.

Microbiology

Dr. Shweta Kumari Junior Resident-2, Department Of Microbiology, DMC, Darbhanga.

Dr. R S Prasad* Associate Professor, Department Of Microbiology, DMC, Darbhanga.
*Corresponding Author

Dr. Ajay Kumar Assistant Professor, Department Of Microbiology, DMC, Darbhanga.

ABSTRACT

BACKGROUND: Emergence of Multidrug resistant Tuberculosis (MDRTB) has become a significant obstacle for Tuberculosis (TB) control. Rifampicin (RIF) resistance is important indicator of MDRTB. Rapid simultaneous detection of Mycobacterium Tuberculosis (MTB) and RIF resistance are very essential for effective disease management. CBNAAT (Cartridge Based Nucleic Acid Amplification Test) also known as Gene Xpert MTB/RIF assay is a novel diagnostic tool for detection of MTB and RIF resistance.
AIMS AND OBJECTIVES: To study the usefulness of CBNAAT for rapid detection of MTB and RIF resistance in MDRTB suspected cases.
Materials and Methods: 1002 sputum samples were collected retrospectively from MDRTB suspected cases including both new cases and previously treated cases. These were processed using Gene Xpert MTB/RIF assay. The results were statistically analyzed.
RESULTS: Out of 1002 sputum samples, Rifampicin resistance was detected in 202 (20.15%) samples and RIF sensitive was detected in 800 (79.84%) samples. Out of 800 rifampicin sensitive cases sputum smear positive in 443(55.37) and negative in 357(44.62). Out of 202 RIF resistant samples, sputum smear were positive in 127 (62.87%) cases and negative in 75 (37.12%) cases.
CONCLUSIONS: Gene Xpert MTB/RIF assay is a good screening tool for diagnosis of MTB and detection of RIF's resistance from MDRTB suspected cases within a shorter period.

KEYWORDS

Mtb, Cbnaat, Rif Resistance,

BACKGROUND:-

Mycobacterium tuberculosis (*M. tuberculosis*) remains one of the most significant causes of death from an infectious agent [1]. Tuberculosis (TB) remains a major public health problem, accounting more than 9.4 million incident cases and 1.7 million deaths every year, worldwide [2]. World Health Organization (WHO) estimates that 4.5 million people are co-infected with Human Immunodeficiency Virus (HIV) and TB globally [1, 2].

The emergence of drug resistance to *M. tuberculosis* has become a significant obstacle for TB control [3]. The emergence and spreading of multidrug (MDR) and extensively (XDR) drug-resistant *M. tuberculosis* complex (MTBC) strains poses significant challenges to TB control [2]. Despite low sensitivity in detection of *M. tuberculosis*, acid-fast staining remains the main diagnostic method in resource-limited settings [4, 5]. Mycobacterial culture is the gold standard and the most sensitive method for TB diagnosis; however, its use in clinical practice is limited due to a slow turnaround time, biosafety requirements, and high cost [4, 5]. In 2011, WHO introduced the wide use of Xpert MTB/RIF assay. It is a fully automated diagnostic molecular test using real-time polymerase chain reaction (PCR) technology to simultaneously detect *M. tuberculosis* and rifampicin resistance mutations in the rpoB gene [6]. The Xpert assay is highly rapid, sensitive and specific in diagnosis of both pulmonary and extra pulmonary TB [7]. Furthermore; it was shown to be cost-effective for TB diagnosis compared to microscopy in low and middle income settings [7].

Moreover, documented data on the prevalence of rifampicin resistant *M. tuberculosis* using the newly endorsed method Gene Xpert in our country is limited. Therefore, the aim of this study was to determine the prevalence and associated factors of rifampicin-resistant *M. tuberculosis* among patient's presumptive for either TB or drug resistant TB (DR TB) in Darbhanga medical college Hospital.

AIMS AND OBJECTIVES:

To study the usefulness of CBNAAT for rapid detection of MTB and RIF resistance in MDRTB suspected cases.

MATERIALS AND METHODS:

1002 sputum samples were collected in the department of micro biology, Darbhanga medical college and Hospital retrospectively from MDRTB suspected cases including both new cases and previously treated cases. These were processed using Gene Xpert MTB/RIF assay. The sample processing was done by switch on the instrument prior to processing the sample, then added by double volume of buffer solution in to sample tube (2:1), Then the sample was incubated for room temperature for 15 mints, in this time when sample was not liquefied then increase the time of incubation, then cartridges was labelled with lab number, then 2.5 ml processed sample added in to cartridge with the help of Pasteur pipette, then clicked the create test menu bar to scan the bar code of the cartridges and typed the sample details, then clicked the stat test button (test will take 2 hrs to complete). Result was displayed on screen. Cartridge was removed and discarded with 5% phenol solution. The results were statistically analyzed.

RESULTS:

Out of 1002 sputum samples, Rifampicin resistance was detected in 202 (20.15%) samples and RIF sensitive was detected in 800 (79.84%) samples. Out of 800 Rifampicin sensitive cases sputum smear positive in 443(55.37) and negative in 357(44.62). Out of 202 RIF resistant samples, sputum smear were positive in 127 (62.87%) cases and negative in 75 (37.12%) cases.[Table-1].

Table:-1

n=1002

RIF(R)	202(20.1%)	
RIF(S)	800(79.8%)	
RIF(R), n=202	**Smear positive sample**	**Smear negative sample**
	127(62.8%)	75(37.1%)
RIF(S), n=800	**Smear positive sample**	**Smear negative sample**
	443(55.3%)	357(44.6%)

DISCUSSION

In the present study, the prevalence of *M. tuberculosis* infection was similar with reports of South Africa (26%) [8], Northern Nigeria (23%) [9] and India (27.6%) [10]. However, it was lower compared to reports in Nigeria (31.4%) [11] and Pakistan (37%) [12]. The lower proportion rate of confirmed *M. tuberculosis* in the present study compared to other studies is due to the fact that we included presumptive cases to identify *M. tuberculosis* while other studies included identified cases of *M. tuberculosis* to check gene Xpert technique. In contrast, it is higher than studies conducted in other parts of India [13]. The discrepancy might be due to difference in methods of detection of *M.*

tuberculosis, community and geographical area.

In this study, the detection rate of *M. tuberculosis* was significantly higher in males than females. Likewise, reports from WHO , and Northeast China supports this finding. The reason for this might be due to social and health seeking behavior difference and higher exposure of males to outer environment, smoking and alcoholism . The highest proportion of Gene Xpert positive *M. tuberculosis* cases were seen in the age group of 18–30 years. This is consistent with previous reports in India . This might be due to more exposure to the outer environment, high work load and wide range of mobility of young people to acquire the TB bacilli as young people have.[14]

In the present study, the proportion of *M. tuberculosis* was significantly higher in presumptive DRTB compared to presumptive TB patients ($P < 0.001$). This might be due to treatment failure and acquiring of resistant bacilli from drug resistant TB contacts. Moreover, significantly higher proportion of *M. tuberculosis* was found among patients treated with anti-TB drugs compared to treatment naïve patients in the present study. This finding was comparable to a study conducted in Zimbabwe [15].

Rifampicin-resistant *M. tuberculosis* is a serious health problem in the study population. The prevalence of rifampicin-resistant *M. tuberculosis* in this study was in keeping with previous studies in Nigeria [17], North India [18], Iran [19] and Northeast China [20]. However, it was higher than studies observed in Ethiopia , Kenya, Nigeria , Uganda and South of Iraq . In contrast, the proportion of rifampicin-resistant *M. tuberculosis* was lower than reports in other parts of Ethiopia and Chile. The variation could be due to difference in risk for HIV acquisition, exposure to anti-TB drugs and national TB control program. The relative higher proportion of rifampicin-resistant *M. tuberculosis* in our study could be due to the use of rifampicin to treat other conditions. Moreover, rifampicin has several adverse effects which could result in patient non-adherence and hence may lead to the selection of resistant strains.

In the present study, the proportion of rifampicin-resistant *M. tuberculosis* was significantly higher among previously treated patients compared to treatment naive patients which might be due to failure from previous treatment and contact with drug resistant TB patients . However, the level of rifampicin resistance among previously untreated cases (6.7%) in the analysis close to the reported prevalence of rifampicin- resistant MTB (10.4%). This finding is a significant relevance in the current global and regional efforts to accurately and timely diagnose MDR-TB with the scale up of molecular technology like Gene Xpert MTB/RIF, providing quick results of rifampicin resistance as a proxy to MDR-TB.

In the present study, the proportion of extra-pulmonary tuberculosis was significantly higher compared to pulmonary tuberculosis; in addition the proportion of rifampicin-resistant *M. tuberculosis* was higher in non-respiratory specimens compared to sputum. This conforms to a study in Cambodia [20]. This demonstrates that rifampicin-resistant extra-pulmonary *M. tuberculosis* infection is a major health problem in resource-limited settings.

The major strength of this study was detection of *M. tuberculosis* and rifampicin resistance using the newly endorsed method Gene Xpert MTB/RIF assay from sputum and non-respiratory specimens. However, the major limitation of this study was determination of the sample size using single population formula which may overwhelm some of the associated factors. This study could not do the level of resistance to other anti-TB drugs and the finding of Gene Xpert was not compared to acid fast bacilli microscopy. Thus the finding of this study should be interpreted with these limitations.

CONCLUSSION:

Rifampicin-resistant *M. tuberculosis* is prevalent both in pulmonary and extra-pulmonary tuberculosis cases in the study area. Previous treatment with anti-TB drugs was significantly associated with rifampicin resistance. The strong association of rifampicin resistance with previous treatment suggests that improved monitoring of treatment to limit the emergence of drug resistant *M. tuberculosis*. Hence, the use of Gene Xpert should be scaled up across the country for rapid diagnosis, management and expanded surveillance of drug-resistant *M. Tuberculosis*

REFERENCES:-

1. Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the Gene Xpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extra pulmonary specimens. J Clin Microbiol. 2011;49(12):4138–4141.
2. Ioannidis P, Papaventsis D, Karabela S, Nikolaou S, Panagi M, Raftopoulou E, et al. Cepheid Gene Xpert 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(8):3068–3070.
3. World Health Organization. Tuberculosis Progress. WHO/HTM/TB 2014. Geneva: WHO; 2015.
4. World Health Organization. Global Tuberculosis Report. WHO/HTM/TB 2014. Geneva: WHO; 2014.
5. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): Global report on surveillance and response. Geneva. WHO/HTM/TB 2010. Geneva: WHO; 2010.
6. Mekonnen F, Tessema B, Moges F, Gelaw A, Eshetie S, Kumera G. Multidrug resistant tuberculosis: prevalence and risk factors in districts of metema and West armachiho Northwest Ethiopia. BMC Infect Dis. 2015;15:461. doi: 10.1186/s12879-015-1202-7
7. Mekonnen D, Admassu A, Mulu W, Amor A, Benito A, Gelaye W, et al. Multidrug and heteroresistant Mycobacterium tuberculosis and associated gene mutations in Ethiopia. Int J Infect Dis. 2015;39:34–38.
8. Tessema B, Beer J, Emmrich F, Sack U, Rodloff AC. First- and second-line anti-tuberculosis drug resistance in Northwest Ethiopia. Int J Tuberc Lung Dis. 2012;16(6):805–811
9. Getahun M, Ameni G, Kebede A, Yaregal Z, Hailu E, Medihn G, et al. Molecular typing and drug sensitivity testing of Mycobacterium tuberculosis isolated by a community-based survey in Ethiopia. BMC Public Health. 2015;15:751.
10. Nigus DM, Lingerew WM, Beyene BA, Tamiru AA, Lemma MT, Melaku MY. Prevalence of multi drug resistant tuberculosis among presumptive multi drug resistant tuberculosis cases in Amhara National Regional State, Ethiopia. J Mycobac Dis. 2014;4:152.
11. Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, et al. Comparison of Xpert MTB/RIF assay and the conventional sputum microscopy in detecting Mycobacterium tuberculosis in Northern Thailand. Tuberc Res Trea. 2015;2015:1–6.
12. Story BE, Helb D. Evaluation of the analytical performance of the Xpert MTB/RIF assay" J Clin Microbiol. 2010;48(7):2495–2501.
13. Chang K, Lu W, Wang J. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis" J Infect. 2012;64(6):580–588
14. Boehme CC, Nabeta P, Hillemann D. Rapid molecular detection of tuberculosis and rifampin resistance" N Eng J Med. 2010;363(11):1005–1015.
15. Cox HS, Mbhele S, Mohess N, Whitelaw A, Muller O, Zemanay Z, et al. Impact of Xpert MTB/RIF for TB diagnosis in a primary care clinic with high TB and HIV prevalence in South Africa: a pragmatic randomised trial. PLOS Med. 2014;11(11):e1001760
16. Aliyu G, El-Kamary SS, Abimiku A, Ezati N, Mosunmola I, Hungerford L, et al. Mycobacterial etiology of pulmonary tuberculosis and association with HIV infection and multidrug resistance in Northern Nigeria. Tuber Res Trea. 2013;2013:1–9.
17. Alvarez-Uria G, Azcona JM, Midde M, Naik PK, Reddy S, Reddy R. Rapid diagnosis of pulmonary and extra-pulmonary tuberculosis in HIV-infected patients. Comparison of LED FluorescentMicroscopy and the GeneXpert MTB/RIF assay in a district hospital in India. Tuberc Res Trea. 2012;2012:1–4.]
18. Dinic L, Akande P, Idigbe EO, Ani A, Onwujekwe D, Agbaji O, Akanbi M, Nwosu R. Genetic determinants of drug-resistant tuberculosis among HIV infected patients in Nigeria. J Clin Microbiol. 2012;50(9):2905–2909
19. Adeniyi B, Wahab M, Lekuk C, et al. Multi-drug-resistant tuberculosis in Northern Pakistan. JPMA. 2004;54:469
20. Deribew A, Negussu N, Melaku Z, Deribe K. Investigation outcomes of tuberculosis suspects in the health centers of Addis Ababa, Ethiopia. PLoS ONE. 2011;6(4):e18614.