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Cross-sectional analysis of Rifampicin resistance in pulmonary tuberculosis at a tertiary care centre

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Abstract
Introduction: - In the ongoing battle against Tuberculosis, the emergence of drug-resistant Tuberculosis posed a significant challenge it to treatment and its control. Drug resistant Tuberculosis is not only difficult to treat, leads to higher morbidity and mortality, higher cost and complexity. According to global tuberculosis report 2022, multidrug resistant rifampicin resistant (MDR/RR-TB) was 3.6% among new cases and 18% among previously treated patients. MDR –TB prevalence in India was 3% and previously treated 35%.
Aim and objectives: - The goal of the current study is to determine the prevalence of Rifampicin resistance among Pulmonary Tuberculosis patients in the Rohilkhand region as well as to compare the diagnostic.
Materials and Methods: - This was a retrospective, observational record-based cross-sectional study conducted from January 2021 to December 2022 in a tertiary healthcare institution situated in the Bareilly, Uttar Pradesh India. The study population was all sputum smear-positive symptomatic patients, all presumptive Pulmonary TB patients and suspected by clinical investigation, visited to the DOT centre during the study period.
Results: - This study comprised 295 sputum samples in which 233 of these were found to be positive and 62 to be negative using fluorescence microscopy. 208 sample had confirm Mycobacterium tuberculosis by GeneXpert MTB/RIF or Cartridge Based Nucleic Acid Test (CBNAAT). While 87 samples were negative. 15 isolated cases of rifampicin resistance which was prevalence rate was (7.22%). According to GeneXpert MTB/RIF, eight patients from previously treated cases and 7 cases from recently identified cases of pulmonary tuberculosis exhibit Rifampicin resistance. The findings indicate that men are more vulnerable than women to have pulmonary tuberculosis.
Conclusion: - In this study prevalence rate of Rifampicin resistance quite lower than the other study. It shows reporting early and awareness about tuberculosis is quit high. So early detection of tuberculosis cases which help in minimize Rifampicin resistance cases in this region with another region of India.

Keywords: Rifampicin resistance, flourescent microscopy, cartridge based nucleic acid amplification test or GeneXpert MTB/RIF assay, mycobacterium tuberculosis, pulmonary tuberculosis

Introduction

In the ongoing battle against Tuberculosis, the emergence of drug-resistant Tuberculosis posed a significant challenge it to treatment and its control. Drug resistant Tuberculosis is not only difficult to treat, leads to higher morbidity and mortality, higher cost and complexity [1]. According to global tuberculosis report 2022, multidrug resistant rifampicin resistant (MDR/RR-TB) was 3.6% among new cases and 18% among previously treated patients. MDR – TB prevalence in India was 3% and previously treated 35% [2, 3].

Thus, India is in a very critical phase with significant burden of drug-resistant Tuberculosis. As such early diagnosis of Tuberculosis and drug sensitivity testing is very crucial [4]. (ponseca) For long the diagnosis of Tuberculosis is being done by sputum smear microscopy by Ziehl-Neelson method & fluorescent microscopy technique which are easy and quick, but lower sensitivity (lower limit of detection is ≥10 [4] are bacteria per ml [5]. It becomes major problem in extra-Pulmonary cases because these samples usually contain small number of organisms. [5] Culture considered as gold standard also suffer from drawbacks like a long time of 6-8weeks to become positive, [6] Limit of detection being $\geq 10^2$ to 10^3 bacteria /ml, with another addition of 4week for drugs susceptibility testing. The newer rapid molecular diagnostic methods like gene-Xpert have offered solution for this problem [7].

In early 2011, the WHO approved a novel, rapid, and automated cartridge based nucleic acid Amplification test (CBNAAT) or Xpert MTB/Rif assay that could detect Mycobacterium tuberculosis and Rifampicin resistance at the same time. It has a detection limit of 5 purified DNA genome copies per reaction (131 colony forming units per mL of sputum) [8]. The only drawback of CBNAAT is that detects both live and dead bacteria, it cannot distinguish between active and cured Tuberculosis [9]. The most important anti Tuberculosis agent is Rifampicin. MDR-TB is represented by Rifampicin resistance (RR) [10].

The Xpert MTB/Rif assay, a fully automated diagnostic molecular test that uses real-time polymerase chain reaction (PCR) technology to simultaneously detect Rifampicin resistance mutation in the rpo B gene, was widely adopted by the WHO in 2011. The Xpert assay can diagnose both Pulmonary and extra-Pulmonary Tuberculosis with extreme speed, sensitivity, and specificity [11].

The goal of the current study is to determine the prevalence of Rifampicin resistance among Pulmonary Tuberculosis patients in the Rohilkhand region as well as to compare the diagnostic [9].

Materials and methods study design, area, and period

This was a retrospective, observational record-based cross-sectional study conducted from January 2021 to December 2022 in a tertiary healthcare institution situated in the Bareilly, Uttar Pradesh India. The study population was all sputum smear-positive symptomatic patients, all presumptive Pulmonary TB patients and suspected by clinical investigation, visited to the DOT centre during the study period.

Inclusion and exclusion criteria

Inclusion criteria: - All presumptive tuberculosis patients, chest radiograph showing any anomalies.

Exclusion criteria: - Extra Pulmonary Tuberculosis patient, cancerous patient, fungal infected patient.

Specimen Collection: - According to the National Tuberculosis Elimination Programme (NTEP), two sputum samples were taken [12, 13].

Specimen Processing: In the case of Pulmonary tuberculosis, two sputum samples were advised: a spot sample (taken under supervision on the same day) and an early morning sample (taken the following day). Alternatively, two spot samples that are at least one hour apart can be taken. Sputum should be at least 2 to 5 ml in volume and ideally mucopurulent. They had been told to brush their teeth and rinse their mouths with clean water to prevent contamination from food and other debris [12].

Using fluorescent microscopy, both sputum samples were analysed if visualised the TB bacilli and not visualised but clinical investigation like (X rays) suspected then patient advice to collect another early morning sputum sample in falcon tube about to 2 -5 ml send for further processing by the GeneXpert MTB/RIF system, or a cartridge-based nucleic acid amplification test (CBNAAT), at the district tuberculosis hospital in Bareilly, Uttar Pradesh. The tests were then carried out on the CBNAAT machine in accordance with the manufacturer's suggested protocols, than CBNAAT report about detection of tuberculosis and Rifampicin resistance taken from nikshay portal site.

A. Phenotypic identification

1. Fluorescent Microscopy.

B. Genotypic identification (Probe based amplification test)

1. Cartridge Based Nucleic Acid Amplification Test (CBNAAT).

Statistical analysis: - The data will be entered to MS Excel where means and standard deviation was calculated.

Ethical consideration: - The study was approved by the university Ethics Committee before commencing the study vide memo no. BIU/REG/PhD/390 dated 26 05, 2022. Individual patient consent was not taken as it was a retrospective record-based study.

Result

This study comprised 295 sputum samples in which 233 of these were found to be positive and 62 to be negative using fluorescence microscopy.

The patient sputum sample 62 which was negative by fluorescent microscopy but clinically investigation of lung shown granuloma by X-ray. Consequently, a sputum specimen is delivered to the District Hospital Bareilly for further analysis to confirm Mycobacterium tuberculosis and Rifampicin resistance employing a molecular method GeneXpert MTB/RIF or Cartridge Based Nucleic Acid Test (CBNAAT). (CBNAAT) was revealed that Mycobacterium tuberculosis was identified in 208 samples, While 87 samples were negative. Tuberculosis positive patient isolated from flourescent positive and negative given in (Table-1) and 15 isolated cases of rifampicin resistance which was prevalence rate was (7.22%). According to GeneXpert MTB/RIF, eight patients from previously treated cases and 7 cases from recently identified cases of pulmonary tuberculosis exhibit Rifampicin resistance. The findings indicate that men are more vulnerable than women to have pulmonary tuberculosis, as seen in fig -2.

However, out of the 295 patients, 194 were newly diagnosed with pulmonary tuberculosis, and 101 possessed the disease but had undergone previous treatment fig-3.

Discussion

This study evaluates the susceptibility and resistance rate of Rifampicin from community acquired Pulmonary tuberculosis patient attending at Rohilkhand Medical College and hospital Bareilly Uttar Pradesh

The study provides valuable laboratory data and allows comparison of the situation of rifampicin resistance at this region.

According to Indian annual report (2022) prevalence rate of multidrug resistance was 9% all over India, Rifampicin resistance rate was 10.4 % by cartridge based amplification test (CBNAAT) and 4.5 % by Truenaat test, Rifampicin resistance estimated death rate was 11 % from Rifampicin resistance rate [14].

In table number no 1 shows difference between fluorescent positive cases and CBNAAT positivity this may because of CBNAAT detect live and dead bacilli and total 131 colony forming unit, CBNAAT negative cases but fluorescent positive because of fluorescent test is very sensitive case it detect non tuberculosis bacilli (mott) also so sensitivity of CBNAAT is high but specifity of fluorescent test is less it not detect only tuberculosis bacilli other non-tuberculosis bacilli.

Our study shows prevalence rate of Rifampicin resistance 7.22% (16/208) by CBNAAT that was more than the other south Indian study Shivekar *et al*., 2020 [15] was 5.4 % (498/2245) and less than other study like Mishra R *et al* 2021 [16] from Lucknow was 23.5 (166/706) Rifampicin mono resistance and Archana *et al*., 2021 [17] 13.7 % (103/754) from Hyderabad. This may be caused by unsuccessful treatment, noncompliance with antituberculosis medication, and contact with a drug-resistant TB patient. Out 16 rifampicin resistance patient, 8 (50%) previously diagnosed and 7 (49%) newly diagnosed. So the variation of previously diagnosed and newly diagnosed was similar, this shows the new individual exposure to resistance patient of presumptive tuberculosis is too high. This shows no awareness of presumptive tuberculosis patient about living activity in household and out house environment taking precaution like cover their

mouth and nose during inhaling and coughing. In this study, the Rifampicin resistance M. Tuberculosis was higher in male than female and the higher proportion of rifampicin resistant MTB were seen in the age group of 21- 30 years (25%) 51-60 (18.6%).

Out of these, 295 patients were diagnosed as TB in GeneXpert MTB/RIF assay in all age groups, of which 194 (65.76%) were males and 101 (34.24%) were females which is similar to other study done in 548 sputum specimens in which 340(62%) samples were of males. (Kumar *et al*., 2019) [18]. this is lowest from this study male 1292 (81.26%) highest for female 298 (18.74%) from west Bengal study (Adhikary *et al*., 2022) [19] about to similar this study male 124 (36.4%), 114 (39.3%) from Agra Uttar Pradesh (Gautam PB *et al*., 2018) [20]. This may be caused by social and health seeking behaviours that are different (such as not eating a complete meal while working), and it may also be due to men being exposed to more environmental hazards like smoking and alcoholism. Majority of study population age group were in age group of 21-30 years which is similar to study from Maharashtra (Deshmukh *et al*., 2021) [21] followed by 51-60 and 41-50 years shown in table 2. The higher proportion of rifampicin resistant MTB were seen in the age group of 21-30 years (25%), 31-40 (19 %) this was too less than Gautam PB *et al* 2018 study (36.7%) shown in table 2.

In current study out of 41 patient of diabetes shows 3 (7.31%) patient of Rifampicin resistance that was lower than other study 13/58 (10.5%).

In current study shows smoker Rifampicin resistance patient was 3/73 (4.10%) that was more lower another study was 5/45 (10.5%) Ali M *et al* 2023 [22].

In our study only only one serotype of HIV found which is similar to Gautam PB 2018 *et al* study with history of contact with tuberculosis affected patient [9].

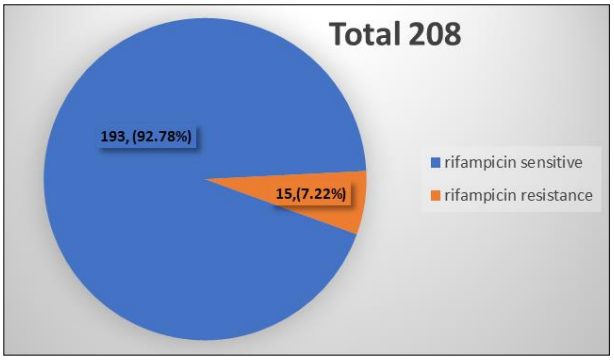

Fig 1: Prevalence rate of Rifampicin

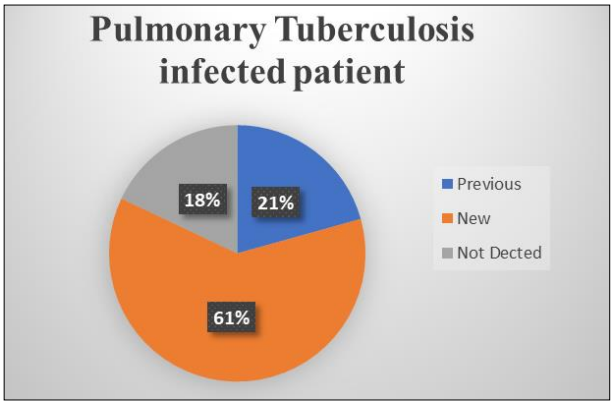

Fig 2: Pulmonary tuberculosis infected patient

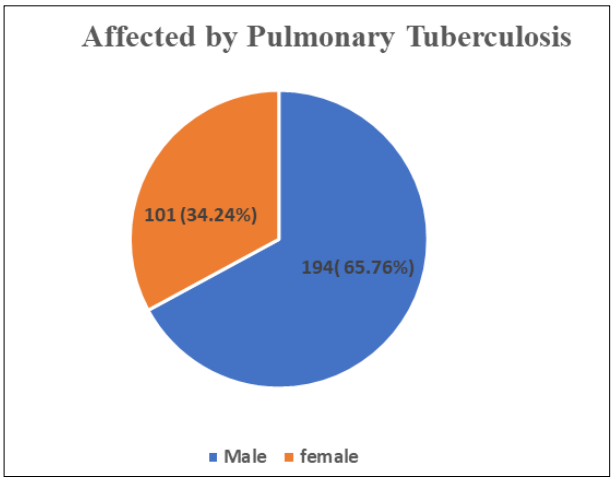

Fig 3: Sex distribution affected by pulmonary tuberculosis

Table 1: Estimated positive cases identified by Fluorescent microscopy and CBNAAT method

Total Positive 295	
Total FM positive 233	Positive by CBNAAT (174)
	Negative by CBNAAT (59)
Total FM negative patient 62	Positive by CBNAAT (34)
	Negative by CBNAAT (28)

Table 2: Sample distribution of sex and age group

Age Group and Sex Distribution								
Age Group	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80
Total	4	44	58	32	56	59	36	6
Male	2	18	27	22	40	48	32	5
Female	2	26	31	10	16	11	4	1

Table 3: Smoker details are as under showing the following table

Smoking Status	Total (FM)	TB Diagnosed (CBNAAT)		Rifampicin Resistance	Rifampicin sensitive
		Positive	Negative		
Smoker	FM 87	73	14	3	70
Non-Smoker	FM 208	142	66	13	195

Table 4: Diabetes associated and Tuberculosis and Rifampicin resistance cases

Total diabetic patient	Mycobacterium detected	Rifampicin resistance	Rifampicin sensitive
49	41 ND (8)	3	38

Table 5: HIV associated Tuberculosis and Rifampicin resistance cases

Total HIV patient	Mycobacterium detected	Rifampicin resistance	Rifampicin sensitive
1	1	0	1

Conclusion

Our study provide burden of Rifampicin resistance prevalence rate in Bareilly region which unrolled at rohilkhand medical college and hospital and also compared with another study lower than other Uttar Pradesh study (like Lucknow, Agra) and more than other state like south region India. This study shows that in Bareilly region presumptive tuberculosis patient quite prevalent.

In this study prevalence rate of Rifampicin resistance quite lower than the other study. It shows reporting early and awareness about tuberculosis is quit high. So early detection

of tuberculosis cases which help in minimize Rifampicin resistance cases in this region with another region of India.
This study provide rough estimate of Rifampicin resistance and also provide information to collaborator to plan and strategy to manage Rifampicin resistance prevalence rate to another region of Uttar Pradesh, India.

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