Prevalence of Rifampicin Resistant Mycobacterium tuberculosis by Genexpert Assay among Presumptive Pulmonary tuberculosis Patients in Dhulikhel Hospital, Nepal

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ABSTRACT

BACKGROUND

Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB) is a preventable disease but the rise of drug-resistant TB has become a major challenge for its control. Rifampicin resistance, a key marker of multidrug-resistant TB (MDR-TB), can be rapidly detected by GeneXpert MTB/RIF assay. The objective of this study was to determine the prevalence of tuberculosis and rifampicin-resistant/multidrug-resistant Mycobacterium tuberculosis among presumptive pulmonary tuberculosis patients in Dhulikhel hospital.

METHODS

A hospital-based cross-sectional study was conducted from May to July 2024 among 345 tuberculosis suspected patients whose sputum sample was processed for GeneXpert MTB/RIF assay using standard procedures. Statistical analyses, including chi-square and logistic regression were performed to assess associations between patient characteristics and TB positivity or rifampicin resistance.

RESULTS

In 345 participants, 30 (8.7%) tested positive for Mycobacterium tuberculosis. Among them, 25 (83.3%) were male and 5 (16.7%) were female. Rifampicin sensitivity was detected in 26 cases (86.7%), resistance in 1 case (3.3%) and indeterminate results in 3 cases (10%). TB positivity was more frequent among older adults (≥40 years), with the highest prevalence in males. The only rifampicin-resistant case was observed in a female aged 15–39 years.

CONCLUSION

The study indicates that TB is a public health problem in Nepal, mostly affecting elderly and male populations. Detection of resistance to Rifampicin indicates the need for timely diagnosis, regular monitoring for drug-resistance and efficient TB control measures. The GeneXpert assay has been proven to be effective in rapidly detecting TB and Rifampicin resistance in low-resource settings.

KEYWORDS

Tuberculosis, Mycobacterium tuberculosis, Pulmonary, GeneXpert, Rifampicin resistance.

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BACKGROUND

Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB) is a preventable and curable illness that primarily affects the lungs but can also affect any other part of the human body.1 It has been a big health issue around the world, and the rise of drug-resistant TB has become a major challenge for controlling the disease effectively. In 2022, 10.6 milllion people were infected with TB worldwide causing 1.3 million deaths, especially affecting HIV patients.2 TB remains a significant public health challenge in Nepal.3 According to the global TB report in 2023, Nepal had about 70,000 people with tuberculosis and 18,000 deaths from the disease.2

Globally, about 410,000 people around the world got multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB).2 One of the primary risk factors for drug-resistant TB is challenges in treatment and diagnosis, particularly prevalent in developing countries. Rifampicin, an important drug in TB management, works by inhibiting RNA polymerase. Resistance is caused by mutation in rpoB gene.4 Rapid detection of Mycobacterium tuberculosis (MTB) is essential for prompt diagnosis and disease management to reduce the risk of transmission and emergence of MDR-TB.3 The WHO endorsed Xpert MTB/RIF assay is a test that simultaneously detects MTB and Resistance to Rifampicin (RIF) caused by mutation to rpoB gene within two hours. GeneXpert is an automated molecular diagnostic tool for performing Xpert MTB/RIF asasy. Rifampicin resistant Pulmonary Tuberculosis (PTB) isolates found by GeneXpert were strong predictors of MDR-TB.6 It has been demonstrated to offer cost-effective tuberculosis diagnosis in resource-constrained nations when compared to microscopy.3

The objective of this study was to determine the prevalence of tuberculosis and rifampicin-resistant/multidrug-resistant Mycobacterium tuberculosis in patients in Dhulikhel hospital. The assessment of resistant patterns will provide significant insights for management and control of TB.

METHODS

This is a hospital-based cross sectional study conducted at Dhulikhel Hospital, Kavre from May to July 2024. The hospital is a tertiary care referral center with high patient flow. The patients with clinical symptoms of TB assessed by clinicians were included.

Sputum samples from all suspected TB patients were collected after they were informed about proper sputum collection techniques. Census sampling technique was used. Prior to collecting the specimen, patients were received instructions on how to collect the sputum sample, and the processing of the sputum sample was done according to the Standard Operating Procedures (SOPs) of the MTB/RIF Xpert assay technique.5 Specimens were processed according to standard operating procedures for the Gene Xpert MTB/RIF assay. Samples were mixed with buffer solution (2:1 ratio)6, incubated for 15 minutes3, and 2 mL of the mixture was loaded into the Gene Xpert cartridge. Results were electronically generated after 90 minutes.7

Ethical approval was obtained from Institutional Review Committee of Kathmandu University School of Medical Sciences (Approval No: 142/24). Informed consent was obtained from all participants. Statistical analysis were done using Stata version 15. Chi square tests and logistic regression were used to assess associations between independent variables and TB/ rifampicin resistance. Results were presented as odds ratio (OR) with 95% confidence intervals (CI). A p-value of <0.05 was considered statistically significant.

RESULT

Out of the 345 study participants, 30 (8.70%) tested positive for MTB (Figure 1). Among which, 25 (83.33 %) were male and 5 (16.67 %) were female (Figure 2). 26 (86.67 %) participants were Rifampicin sensitive, 1 (3.33 %) were resistant and 3 (10.00 %) were indeterminate (Figure 3).

Out Of the total 345 participants, 14 (4.06%) were less than 15 years, 58 (16.81%) were between 15-39 years, 76 (22.03%) were between 40-59 years and 197 (57.10%) were above 60 years (Table 1). In the age-wise distribution of participants, MTB detection was highest in older age groups ie.40+, particularly in males. Detection was absent in those under 15 years. Overall, a significant number of MTB positive cases were found among the elderly (Table 1).

All of the participants showing rifampicin resistant were female (Table 2). In age-wise distribution of all positive cases, 6 (20.00%) were aged group 15-39 years, 12 (40.00%) from the aged group 40-59 years, 12 (40.00%) from the aged group 60 years and above (Table 2).

The majority of MTB cases (26 out of 30) were sensitive to rifampicin, with the highest sensitivity observed in males aged from 40-59 and above 60 years. Only one case showed rifampicin resistant, occurring in a female aged between 15-39 years. Three cases were indeterminate, primarily in males aged more than 40 years (Table 2).

DISCUSSION

In this study, we have assessed the prevalence of MTB and RR-TB among 345 presumptive TB cases using Gene Xpert MTB/RIF assay. The rate of positivity was 8.7% with significantly predominant male cases (83.33%). Among the confirmed MTB cases, 3.33% were Rifampicin resistant. The prevalence of MTB mirrors the findings from similar studies (9%-15%) conducted in resource-limited settings.3,5,8 Slightly lower prevalence in our study may be due to improved health measures or demographic differences.

Disproportionate occurrence of TB between genders is still evident. The higher prevalence of male aligns with global data and maybe attributed to factors like occupational exposure, smoking, alcohol consumption and delayed health seeking behavior in men.2,9 The only RR-MTB case was observed in a female patient although there was lower number of MTB positive females than males indicating the need for strict monitoring of resistance patterns in both the genders. The higher TB positivity rate among older adults (aged 40 years and above) reflects decline to immunity with age which in turn can lead to higher susceptibility to the disease and higher rate of reactivation of latent infection.2

The prevalence rate of RR-MTB in our study is 3.33%. This rate is similar to other studies done in South Asia and Sub-Saharan Africa, though this slightly lower rate might not reflect the true burden of RR-MTB patients.3,5,6,10 Patients with indeterminate rifampicin results (which was 10% in our study) or patients with inadequate sputum quality were not further investigated. Indeterminate results and inadequate sputum quality are linked to low bacterial load. Hence, there should be repetition of the test with proper sample or other confirmatory tests should be done.11

The rapid turnaround time and high specificity of Gene Xpert assay makes it a valuable diagnostic tool for detecting TB and resistance to Rifampicin. However, it may sometimes show indeterminate results and cannot detect resistance to other drugs except Rifampicin due to which other diagnostics tests like culture based drug-susceptibility testing and whole genome-sequencing may be required.4,5,12 Although the limitations like single center study, small sample size and comparatively shorter study period prevail, the findings in this study provides insights to burden of TB and RR-TB in Nepal indicating the need of further monitoring, better diagnostic tools and focused intervention in high-risk groups.

CONCLUSIONS

The study points out to the fact that Tuberculosis still contributes to a significant health burden in Nepal with 8.7% of positivity among presumptive TB patients. Though Rifampicin resistance was detected only in 3.3% of MTB positive cases, it still possess a significant risk and stresses on the importance of timely detection and effective treatment regimens in the patients. The higher positivity among old age-groups and predominance of male indicates the importance of targeted diagnostic methods and strategic treatment and prevention measure. Due to the rapid and accurate results that Gene Xpert provide, it has proven to be an important diagnostic tool for detection of TB and Rifampicin resistance in TB endemic areas.

Figure 1. Distribution of study participants.

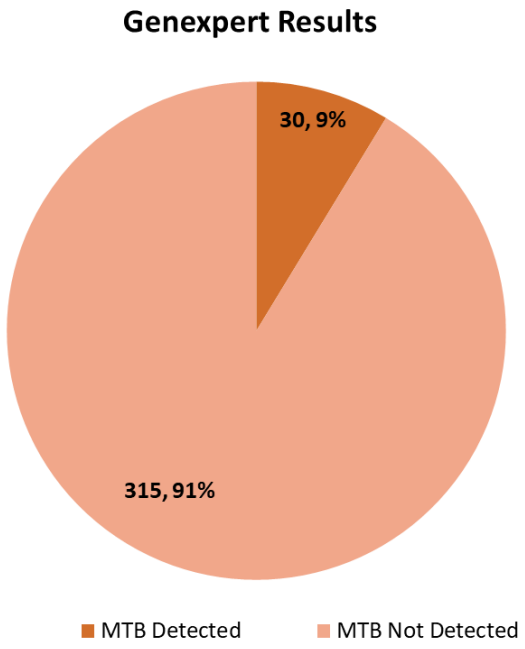

Figure 2. Gender-wise distribution of the Positive participants for MTB

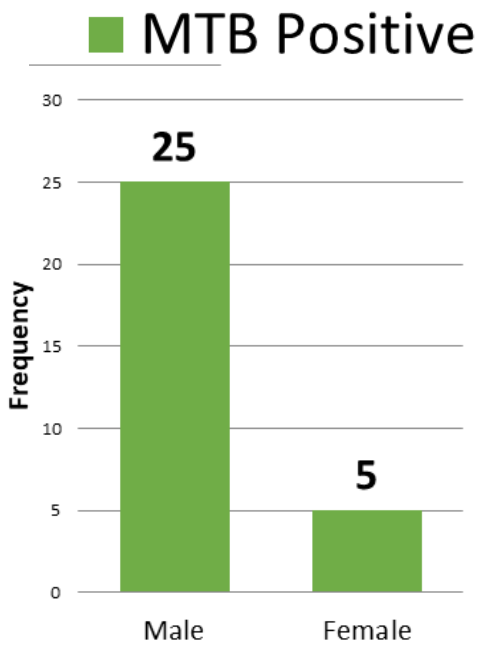

Figure 3. Distribution of Rifampicin sensitivity to MTB

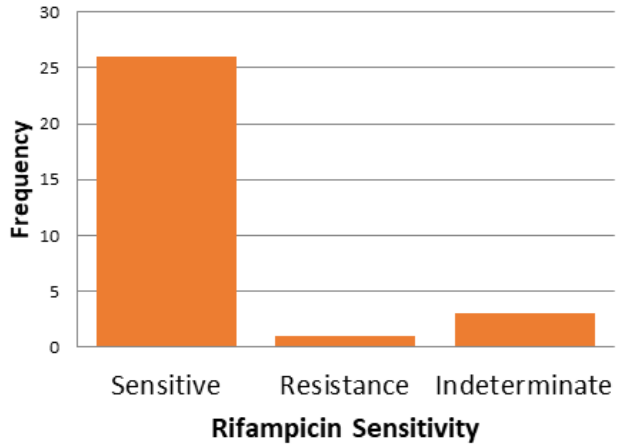

Table 1. Frequency of positive results in relation to Age and Gender

	MTB Not Detected (n=315) n (%)		MTB Detected (n=30) n (%)		Total (n=345) n (%)
Age (In Years)	Male	Female	Male	Female	
<15	9 (5.70)	5 (3.19)	0 (0.00)	0 (0.00)	14 (4.06)
15-39	28 (17.72)	24 (15.29)	3 (12.00)	3 (60.00)	58 (16.81)
40-59	23 (14.55)	41(26.11)	11 (44.00)	1 (20.00)	76 (22.03)
≥60	98 (62.03)	87 (55.41)	11 (44.00)	1 (20.00)	197 (57.10)

Table 2. Frequency of MTB resistance to rifampicin in relation to Age and Gender (n=30)

	Sensitive (n=26) n (%)		Resistance (n=1) n (%)		Indeterminate (n=3) n (%)		Total (n=30) n (%)
Age (In Years)	Male	Female	Male	Female	Male	Female	
<15	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
15-39	3 (13.65)	2 (50.00)	0 (0.00)	1 (100.00)	0 (0.00)	0 (0.00)	6 (20.00)
40-59	10 (45.45)	1 (25.00)	0 (0.00)	0 (0.00)	1 (33.33)	0 (0.00)	12 (40.00)
≥60	9 (40.90)	1 (25.00)	0 (0.00)	0 (0.00)	1 (33.33)	0 (0.00)	12 (40.00)

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