Drug Susceptibility Pattern of Mycobacterium Tuberculosis Isolated from New Cases of Pulmonary Tuberculosis at a Tertiary Care Hospital of Eastern Nepal

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ABSTRACT

Introduction: Multi drug resistant tuberculosis (TB) possesses a serious threat for the control of the *Mycobacterium tuberculosis* isolated from newly diagnosed cases of pulmonary tuberculosis and determine the prevalence of drug resistant tuberculosis in eastern Nepal.

Methods: A cross sectional study was conducted in Department of Microbiology, B.P Koirala Institute of Health Sciences, Dharan, Nepal. A total of 58 smear positive sputum samples were processed for culture in Lowenstein Jensen media and drug susceptibility testing was performed by conventional proportion method using standard protocol. The Chi square test was used and *p* value <0.05 was considered statistically significant.

Results: Among the 58 patients enrolled in the study, the mean age of the patients was 40.7 years with male preponderance of 70.7%. The prevalence of drug resistant TB among culture positive pulmonary TB cases was 23.1% and the rate of multidrug resistant TB was found to be 5.8% in our study. The highest rate of resistance of mycobacterium tuberculosis was towards isoniazid 15.4% followed by rifampicin 13.5%.

Conclusion: The high prevalence of drug resistant TB in our study reflects the improper implementation of tuberculosis control programme in Nepal. Therefore, it emphasizes the need for molecular method for TB diagnosis and effective TB control program in the country.

Keywords: Drug susceptibility testing; Isoniazid; Pulmonary tuberculosis; Rifampicin

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INTRODUCTION

Tuberculosis (TB) is a one of the deadliest communicable disease caused by Mycobacterium tuberculosis (MTB). TB is a global public health problem with a worldwide incidence of 10.4 million cases with 490,000 multidrug-resistant TB (MDR-TB) cases which is defined as resistance to at least isoniazid (INH) and rifampicin (RIF). Around 56% of the estimated cases were from India, Indonesia, China, Philippines and Pakistan.[1]

The worldwide prevalence of MDR-TB is estimated as 3.5% of the new cases and 20.5% of the previously treated cases. The neighbouring countries, India and China have the high burden of TB, accounting for the highest MDR-TB rates. TB is the 6th leading cause of death in Nepal, with 31,764 new TB cases registered in National Tuberculosis Programme, Nepal in 2016.[2] The latest drug resistance surveillance of 2011 in Nepal found the prevalence of MDR-TB to be 2.6% and 17.6% among new and previously treated TB cases respectively among which 8% of the cases were found to be extensively drug resistant tuberculosis (XDR-TB) which is defined as resistant to isoniazid and rifampicin plus any fluoroquinolone and at least one of three second line injectable drugs (amikacin, kanamycin or capreomycin).[2] Lack of effective TB control programme in the country, illiteracy, poverty, overcrowding and migration from high MDR-TB prevalence areas are the major reasons for increasing prevalence of drug resistant TB in populations.[3, 4] Therefore, the objective of this study was to know the drug susceptibility pattern of *Mycobacterium tuberculosis* in newly diagnosed cases of pulmonary TB and determine the prevalence of the multidrug resistant tuberculosis in eastern Nepal.

METHODS

This was a cross-sectional descriptive study conducted over a duration of six months (15th January 2015 to 15th July 2015) in Department of Microbiology, B.P. Koirala Institute of Health Sciences, Dharan, Nepal. The ethical clearance for the study was obtained from Institutional Ethical Review Board. Smear positive sputum samples from 58 new pulmonary tuberculosis patients who gave written informed consents were included in the study.

All the specimens were decontaminated and processed for culture as per standard protocol.[5]

Lowenstein Jensen (LJ) media was used for the primary isolation of Mycobacterium tuberculosis. The MTB isolates were identified on the basis of the colony morphology, acid fast staining, pigment production and no growth on p- Nitro benzoic acid. Drug susceptibility testing (DST) of Mycobacterium tuberculosis was performed for rifampicin (40 ug/ml) and isoniazid (0.2 ug/ml) by proportion method as per the standard guidelines.[6] Mycobacterium tuberculosis H37Rv isolate, was used as a reference control strain for both culture and drug susceptibility testing.

Statistical analysis

Data were analyzed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 16. The Chi square test was used and *p* value <0.05 was considered statistically significant.

RESULTS

A total of 58 smear positive sputum samples were collected and processed for culture and drug susceptibility testing. The age of the patients involved in the study ranged from 14-77 years with mean age being 40.7 years. The highest percentage 72.4% (p <0.05) of the patients belonged to the productive age group i.e. 16-45 years. Out of 58 smear positive TB cases, 41 (70.7%) were male and 17 (29.3%) were female. The age and gender distribution of samples is presented in Table 1.

Table 1: Age and Gender wise distribution of the sputum samples

Age	Male	Female	Total
0-15 YEARS	0	1	1 (1.7)
16-30 YEARS	14	4	18 (31)
31-45 YEARS	14	10	24 (41.4)
46-60 YEARS	7	1	8 (13.7)
>61 YEARS	6	1	7 (12)
TOTAL	41	17	58 (100)

Out of 58 smear positive sputum samples cultured in LJ media, growth was detected in 52 (89.6%) samples. Contamination was observed in 5 (8.6%) samples while one sample was not able to grow in LJ media. With reference to microscopy as standard, the sensitivity of detection of tubercle bacilli was calculated as number of positive growths yielded and was found to be 89.6%. A total of 22 (42.4%) samples

had growth on the 28th day and almost 96.2 % of samples had culture positive on the 42nd day. The mean detection time for culture was 32.7 days.

Among 58 sputum samples processed and cultured in LJ media, growth was observed in only 52 sputum samples. Therefore, drug susceptibility testing and analysis of only those 52 samples could be done. The mycobacterial isolates grown on LJ media were subjected to drug susceptibility testing by proportion method in LJ media. The figure of *M. tuberculosis* growth in LJ culture and DST is depicted in Figure 1.

Out of 52 MTB isolates, 76.9% (n=40) were susceptible to both the drugs and 23.1% (n=12) were resistant to any drugs. The rate of resistance towards rifampicin was 13.5% (n=7), isoniazid was 15.4% (n=8) and 5.8% (n=3) were found to be MDR-TB. The mean turnaround time for DST by PM for 52 samples was found to be 39.3 days. The total mean turnaround time from primary isolation of Mycobacterium tuberculosis to result of drug susceptibility testing was found to be 72 days. The summary of anti-TB drug susceptibility testing is depicted in Table 2.

Table 2: Analysis of drug susceptibility testing of *Mycobacterium tuberculosis* by proportion method

| Description	Number	Percentage
Total Mycobacterium tuberculosis isolates	52	100
Sensitive to both drugs	40	76.9
Resistant to any drugs	12	23.1
Resistant to rifampicin	7	13.5
Resistant to rifampicin only	4	7.7
Resistant to isoniazid	8	15.4
Resistant to isoniazid only	5	9.6
Resistant to both drugs (MDR-TB)	3	5.8
Average duration for DST (days)	39.3	
Average duration for culture + DST (days)	72	
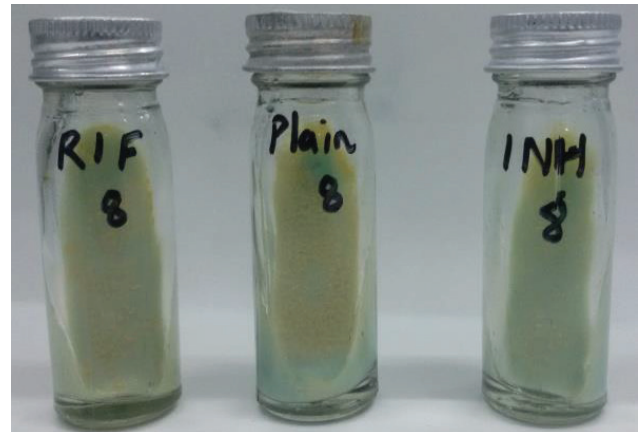
Figure 1: Conventional Proportion Method (Left: Rifampicin Resistance, Middle: Growth Control, Right: Isoniazid Sensitive)

DISCUSSION

A total of 58 smear positive samples from new pulmonary TB patients were included in this study. The age of the patients involved in the study varied from 14-77 years with mean age being 40.7 years. The highest proportion (72.4%) of the patients belonged to economically active age group of 16-45 years. Gender wise distribution of sample showed male preponderance (70.7%) in the study compared to female (29.3%), thereby making male to female ratio of 2.4: 1. There is a strong association between gender and prevalence of tuberculosis in eastern Nepal. This findings are consistent with reports of Bhatta *et al*, who reported that around 73% of the patients belonged to age group 21-50 years with male predominance of 64%.[7] Higher tuberculosis notification rates in economically productive age group could be due to nature of job and physical ability to seek health care facilities. Male preponderance among the study population could be due to higher exposure of men to predisposing factors like smoking, alcoholism and drug abuse.[7]

In our study, among 58 smear positive sputum samples growth was detected in 52 (89.6%) samples while contamination was observed in 5 (8.6%) samples which was consistent with the findings of Chaiyasirinroje *et al*, who documented 10.9% of contamination rate in solid culture media.[8] In this study, 76.9% of the mycobacterium tuberculosis isolates were sensitive to both the drugs and 23.1% of the isolates were resistant to any of the two drugs. The resistance rate for rifampicin was found to be 13.5% and isoniazid 15.4% whereas 5.8% isolates

were reported to be MDR-TB. In accordance to our finding, Massi *et al* documented 60.9 % of the total *M. tuberculosis* isolates to be sensitive to all four drugs, while 39.1 % of the isolates to be resistant to at least one of the four drugs.[9] Similarly, Pradhan *et al* reported the prevalence of MDR-TB to be 4.2 % among new cases and 15.5% among previously treated cases and Khunjeli *et al* documented the prevalence of primary drug resistant tuberculosis to be 4.8 %.[10, 11] However, in 2014, WHO had estimated only 2.2% of all new pulmonary TB cases and 15% of all retreatment cases to be MDR –TB.[2]

WHO estimates that 96% of MDR-TB cases are not being diagnosed and treated properly and only 2% of new cases are tested for drug susceptibility globally.[12] Therefore, the variation in drug susceptibility patterns of MTB isolated in different studies in Nepal could be due to lack of provision of routine drug susceptibility testing among new cases and unavailability of such laboratories in most part of our country where only 0.3 DST laboratories exists per 5 million populations.[13] Over the years, there has been gradual increase in primary MDR-TB in Nepal which may be attributed to the increased rate of the acquired resistance contributing to the rise in primary drug resistant TB. The main causes of high rates of drug resistance tuberculosis in Nepal could be untimely diagnosis and inappropriate treatment of MDR-TB. So, for effective treatment and control of MDR-TB, it is necessary to have the laboratories with sufficient resources which can detect MDR-TB in developing countries like Nepal, where DST facility are not sufficiently available. Therefore, there is a need of molecular methods like GeneXpert MTB/RIF assay which proved to be a promising tool for early diagnosis, appropriate treatment and control of MDR-TB in Nepal.

CONCLUSION

Our study reported high prevalence (23.1%) of drug resistant TB among new cases of pulmonary tuberculosis in eastern Nepal. The drug susceptibility testing of all new cases should be done for proper management and reduction in disease transmission especially in countries with high TB burden. Therefore, it emphasizes the need of molecular methods like GeneXpert MTB/RIF assay for rapid diagnosis of drug resistant TB which may be helpful in control of DR-TB in Nepal.

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