ORIGINAL ARTICLE

Drug resistance pattern of Mycobacteria isolated from smear positive cases of pulmonary tuberculosis in eastern part of Nepal

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ABSTRACT

Introduction: The emergence of drug resistance in tuberculosis has become a major challenge for effective control of tuberculosis. This study was done with an aim to determine the drug resistance pattern of mycobacteria isolated from pulmonary tuberculosis.

Methods: Sputum samples were collected from patients attending B.P. Koirala Institute of Health Sciences, Dharan during 1st June 2006 to 30th May 2007. The smear positive samples were cultured on Lowenstein Jensen medium and drug sensitivity was performed by proportion method.

Results: Among 202 Mycobacterial isolates, 96 (47.52%) were sensitive to all four drugs, 50 (24.75%) were resistant to single drug and 15 (7.42%) were MDR strains. Higher rate of MDR was seen in previously treated cases (15.51%) than in new cases (4.6%).

Conclusion: This study reflects a high burden of drug resistant tuberculosis in the eastern part of Nepal.

Key words: Drug resistance, mycobacterium, pulmonary tuberculosis.

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INTRODUCTION

Tuberculosis (TB) is a disease of poverty and its major impact is in developing countries.[1] According to World Health Organization (WHO), one third of the world's population (2 billion) is infected with *Mycobacterium tuberculosis* with 9 million new cases and 1.4 million deaths in the year 2011.[2] In Nepal, about 45% of the total population is infected with TB, and about 50,000 new cases occur every year, out of which 22,000 people develop pulmonary disease and nearly 10,000 people die every year.[3,4]

Drug-resistance in TB has emerged since the early days of introduction of anti-tubercular therapy. Moreover multidrug-resistant tuberculosis (MDR-TB) has been an area of growing concern, and is posing threat to the efforts of TB control.[5] Further, MDR-TB patients require treatment with more toxic second line drugs and remain infectious for longer period than patients infected with drug sensitive strains.[6] There is paucity of data reflecting the exact burden of drug resistance in the country as the diagnosis is primarily based on sputum smear examination. We conducted this study to determine the drug resistance pattern of mycobacteria isolated from patients with pulmonary tuberculosis in the eastern region of Nepal.

METHODS

This is a cross-sectional study conducted at B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal for a period of one year (1st June 2006 to 30th May 2007).Three sputum samples from the patients suspected of tuberculosis were collected in a wide mouthed plastic container. After the samples were received, smears were prepared and stained by Ziehl-Neelsen staining method.[7] Informed consent was taken from all the patients with AFB positive result. Patient's demographic details and history of previous treatment for tuberculosis were also recorded. One smear positive sample with the highest sputum grade from each patient was used for culture. Homogenization and decontamination was done with 4% NaOH by Petroff's method. The centrifuged sediment was inoculated in two slopes of Lowenstein - Jensen (LJ) medium. The inoculated L-J media were incubated at 37^{o}C and observed on 3rd and 7th day to detect contamination and/or fast growth of atypical mycobacteria. The inoculated media were observed every week to note the growth and morphology of the colony. The contaminated samples were discarded as detected and negative cultures were discarded after 8 weeks.[8]Drug susceptibility test (DST) was done by proportion method. Each culture isolate was tested against four anti tubercular drugs at the following concentration: Isoniazid (INH) 0.25 µg/ml, Rifampicin (RMP) 40 µg/ml, Ethambutol (EMB) 2 µg/ml and Streptomycin (SM) 4 µg/ml.[9] Multi drug resistance (MDR) was defined as tubercle bacilli resistant to both isoniazid and rifampicin with or without resistance to other drugs and poly drug resistance(PDR) was defined as tubercle bacilli resistant to more than one drugs without simultaneous resistance to both isoniazid and rifampicin. Statistical analyses were done by SPSS version 15.0.

RESULTS

A total of 215 sputum smear positive samples from different patients were included in this study. Out of these, 202 (94%) samples yielded Mycobacterium spp, 3 (1%) were culture negative and 10 (5%) cultures were contaminated. Among the culture positive samples, 136 were obtained from males and 66 from females. The age range varied from 7 years to 83 years. The result showed that highest number of culture positive age group was from 31-60. A total 106 isolates were drug resistant strains, 71 of them were isolated from male patients and the remaining from female patients. (Table 1)

Table 1: Age and Sex wise distribution of culture positive patients with drug susceptibility status

Age group (years)	Male			Female			Total (%)
	Resistant (%)	Sensitive (%)	Total (%)	Resistant (%)	Sensitive (%)	Total (%)	
<15	0 (0)	2 (33.3)	2 (33.3)	1 (16.7)	3 (50.0)	4 (66.7)	6 (100)
15-30	21 (28.4)	23 (31.1)	44 (59.4)	16 (21.6)	14 (18.9)	30 (40.5)	74 (100)
31-60	35 (37.6)	30 (32.2)	65 (69.9)	16 (17.2)	12 (12.9)	28 (30.1)	93 (100)
>60	15 (51.7)	10 (34.5)	25 (86.2)	2 (6.9)	2 (6.9)	4 (13.8)	29 (100)
Total	71 (35.1)	65 (32.2)	136 (67.3)	35 (17.3)	31 (15.3)	66 (32.7)	202 (100)

Out of 202 Mycobacterium spp isolates tested for DST against first line drugs, 96 (47.52%) were sensitive to all four drugs. Fifty isolates (24.75%) showed resistance to single drug. This type of resistance was highest with Streptomycin and least (0) with Ethambutol. Poly drug resistance was seen in 41 (20.29%). MDR was seen in 15 (7.42%) of the strains.

Among the total Mycobacterium spp isolated, 71.3% were isolated from new cases and 28.7% were from previously treated cases. Among the isolates form new cases, 35 (24.3%) were resistant to single drug. A total of 26 (15.84%) strains were found to be poly drug resistant. This type of resistance was most common with SM + RIF (10.41%) followed by SM + INH (6.25%). Six (4.16%) strains were MDR strains. Among the isolates form previously treated cases, 15 (25.86%) were resistant to single drug. Poly drug resistance was seen in 15 (25.86%) strains among which SM + RIF was the most common (20.68%). Nine (15.51%) were MDR strains and 4 (6.89%) were resistant to all four anti tubercular drugs tested. Table 2

Table 2: Drug resistance profile of Mycobacteria

Drug Susceptibility testing Result	New Cases		Previously treated Cases		Total
	Number (N)	%	Number (N)	%	
Total tested	144	100	58	100	202
Total Sensitive	77	53.47	19	32.75	96
Total Resistance	67	46.53	39	67.24	106
Monodrug resistance					
SM only	27	18.75	10	17.24	37
INH only	2	1.38	2	3.44	4
RIF only	6	4.16	3	5.17	9
EMB only	0	0	0	0	0
Resistance to 2 drugs					
SM + INH	9	6.25	3	5.17	12
SM + RIF	15	10.41	12	20.68	27
SM + EMB	1	0.69	0	0	1
INH + RIF	1	0.69	1	1.72	2
Resistance to 3 drugs					
SM + INH + RIF	5	3.47	4	6.89	9
SM + RIF + EMB	1	0.69	0	0	1
Resistance to all 4 drugs					
SM + INH + RIF + EMB	0	0	4	6.89	4
MDR	6	4.16	9	15.51	15
Any SM Resistance	58	40.27	33	56.89	91
Any INH Resistance	17	11.8	14	24.13	31
Any RIF Resistance	28	19.44	24	41.37	52
Any EMB Resistance	2	1.37	4	6.89	6

DISCUSSION:

In this study, the male: female ratio among culture positive patients was found to be 2.06:1. This finding is comparable to the national data which shows that male: female ratio of tuberculosis as 2:1. The reason for this gender bias might be because men are more frequently exposed to infection or, woman have less access to health care services than men.[10] The highest number of tuberculosis patient belonged to age group 31-60 in this study while in a similar study

conducted at National Tuberculosis Centre (NTC), highest number of cases belonged to the age group 21-30. [11]

The study revealed that more than half (53.48%) of the isolates were resistant to one or more drugs. Among monodrug resistant strains, Streptomycin resistance (74%) was found to be the highest. Similar result has been reported from a study done at NTC in 2003 in which resistance to streptomycin was highest (60.65%).[12]Streptomycin resistance is a concern as it is an essential component of category II DOTS regimen.[13]

In this study, drug resistance among new cases was found to be 46.53% while drug resistance in previously treated cases was 67.24%. Similar finding has been reported in a study in which 36.84% isolates were drug resistant in new cases and 75.16% isolates were drug resistant in previously treated cases.[12]

Our study also showed that MDR strains were higher in previously treated cases (15.51%) compared to new cases (4.16 %). In a similar study conducted at Tribhuvan University Teaching Hospital in 2000, MDR in new cases was 8.6% while 4 out of 4 (100%) strains were MDR among previously treated patients.[14] while Drug Resistance Surveillance conducted in 2007 has shown prevalence of MDR among new cases to be 2.9% and previously treated cases to be 11.7%.[15]A preliminary unpublished data of DRS carried out by GENETUP in 2011 has shown slight decline of MDR prevalence among new cases (2.6%) but a rather sharp increase (17.6%) in previously treated cases.[16]

CONCLUSION:

This study reflects high burden of drug resistant tuberculosis in both new and previously treated patients in eastern part of Nepal. Culture and drug susceptibility test should be made available with first line anti-tuberculosis drugs in all smear positive patients, and appropriate drugs should be selected for treatment to prevent spread of drug resistant tuberculosis in the community.

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