Drug susceptibility patterns of the *Mycobacterium tuberculosis* isolated from previously treated and new cases of pulmonary tuberculosis at German-Nepal tuberculosis project laboratory, Kathmandu, Nepal

Gobinda Thapa¹, Narayan Dutt Pant²*, Saroj Khatiwada³, Binod lekhak¹ and Bhawana Shrestha⁴

**Abstract**

**Background:** Multidrug resistant tuberculosis (MDR-TB) is a serious public health problem in Nepal. It is a major obstacle for the control of the tuberculosis. The main objectives of this study were to determine the prevalence of the multidrug resistant pulmonary tuberculosis and to evaluate the drug susceptibility patterns of *Mycobacterium tuberculosis* isolated from previously treated and newly diagnosed cases of pulmonary tuberculosis.

**Methods:** A cross-sectional study was conducted from March 2013 to August 2013 at German-Nepal tuberculosis project (GENETUP) laboratory, Kathmandu, Nepal. For this the sputum samples from total of 153 (49 new and 104 previously treated) suspected pulmonary tuberculosis patients were used. The diagnosis of the tuberculosis was performed by using fluorescent microscopy and culture, while the drug susceptibility testing of *Mycobacterium tuberculosis* was performed by proportion method. Lowenstein-Jensen (L-J) medium was used for the culture of *Mycobacterium tuberculosis* and the colonies grown were identified on the basis of the colony morphology, pigment production and biochemical characteristics.

**Results:** The prevalence of MDR-TB among all the cases of culture positive pulmonary tuberculosis was 15.6 %. The rate of MDR-TB among previously treated culture positive tuberculosis patients was 19.4 % and that among newly diagnosed culture positive pulmonary tuberculosis cases was 7.1 %. The highest rate of resistance of *Mycobacterium tuberculosis*, was toward streptomycin (24.4 %) followed by isoniazid (23 %), rifampicin (17.8 %) and ethambutol (15.6 %). Among the total of MDR-TB cases among previously treated patients, highest percentage of the cases were relapse (61.1 %) followed by chronic (16.7 %).

**Conclusions:** The high prevalence of DR/MDR-TB in our study reflects poor implementation of tuberculosis control program. On the basis of the drug susceptibility patterns of *M. tuberculosis* we found in our study, we recommend to include ethambutol instead of streptomycin in the multidrug therapy for the treatment of tuberculosis patients in Nepal. Further, due to high rate of MDR-TB among previously treated patients, we do not recommend to use first line drugs for the treatment of pulmonary tuberculosis among previously treated patients.

**Keywords:** Mycobacterium tuberculosis, Drug susceptibility testing, Multidrug resistant tuberculosis, Pulmonary tuberculosis, Nepal
Background
Tuberculosis (TB) is a communicable disease caused by Mycobacterium tuberculosis. Primarily, the Mycobacterium tuberculosis affects the lungs, causing pulmonary tuberculosis (PTB) but it may also be involved in causing extra pulmonary tuberculosis, in which sites of the body other than lungs are affected. The common mode of transmission of the tuberculosis is through droplet nuclei formed during coughing, sneezing, and speaking of the patients suffering from pulmonary tuberculosis [1].

Tuberculosis is a global public health problem with a worldwide incidence of 9 million TB cases, with 440,000 multidrug-resistant TB (MDR-TB) cases [2]. MDR-TB is defined as the TB caused by strains of Mycobacterium tuberculosis, which are resistant to two first-line drugs: isoniazid and rifampicin [1].

The rate of death due to tuberculosis is highest in Sub-Saharan Africa and Southeast Asia, and the majority (99%) of the death due to tuberculosis occurs in developing countries [3]. It has been estimated that every year about 9 million new TB cases are diagnosed globally [4]. Worldwide, around 1.4 million deaths occurred in 2011 due to tuberculosis and according to World Health Organization (WHO), if proper control measures are not strengthened, by 2020 another 1 billion people will be infected with around 36 million deaths [4].

In Nepal, about 45% of the total population is infected with Mycobacterium tuberculosis (both latent and active infection) [5]. The rapid spread of HIV/AIDS and the recent emergence of multidrug-resistant (MDR) TB may be responsible for worsening impact of tuberculosis and increasing numbers of the tuberculosis cases [6].

The worldwide prevalence of the MDR-TB is estimated to be 5%; with 3.5% of the new cases and 20.5% of the previously treated cases being MDR-TB [7]. The neighboring countries, China and India are two countries with high TB burden, accounting for around one third of the total TB cases in the world and have highest MDR-TB rates [2, 7]. Recently, there have been reports of increasing prevalence of MDR-TB [8]. 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 cases respectively [6]. Recently, the detection of MDR-TB in national surveillance is more dependent on GeneXpert technology [9]. The GeneXpert technology is a rapid and less labor intensive method with low biohazard risk [10]. In contrast the proportion method used in our study is time consuming, more labor intensive (requiring skilled manpower) and has high biohazard risk.

In this study, we determined the prevalence of the multidrug resistant pulmonary tuberculosis and evaluated the drug susceptibility patterns of Mycobacterium tuberculosis isolated from previously treated and newly diagnosed cases of pulmonary tuberculosis. This study presented the more up to date information about the prevalence of MDR-TB and drug susceptibility patterns of Mycobacterium tuberculosis in Nepal.

Methods
Study design
A cross sectional descriptive study was carried out from March 2013 to August 2013 at German-Nepal tuberculosis project (GENETUP) laboratory, Kathmandu, Nepal. For this the sputum samples from total of 153 (49 new and 104 previously treated) suspected pulmonary tuberculosis patients (having clinical and/or radiological features of tuberculosis), received from all over Nepal were used. The specimens which were culture negative and/or contaminated during culture were excluded from the study.

Sample collection
Three early morning sputum samples (per patient) were collected in a sterile, leak proof, wide mouthed, transparent plastic container. Saliva and nasal secretions were not accepted.

Microscopy
For microscopy auramine-phenol fluorochrome staining was used [11].

Sputum culture for Mycobacterium tuberculosis
Method devised by Kent and Kubica was used for the digestion, decontamination and concentration of sputum samples [12]. For culture L-J medium was used. The colonies grown were identified on the basis of the colony morphology, acid fast staining, pigment production and biochemical characteristics [13].

Drug susceptibility testing by proportion method
Drug susceptibility testing of Mycobacterium tuberculosis was performed by proportion method as suggested by Heifets [14].

Quality control
For quality control, M. tuberculosis H37Rv was inoculated into a series of drug containing media and control media and incubated along with media inoculated with the test strains.

Statistical analysis
Data were analyzed using statistical package for the social sciences (SPSS) version 16.0. The $\chi^2$ test was used and $p$-value < 0.05 was considered statistically significant.

Results
Among the total of 153 patients, sputum samples from 129 patients were smear positive and those from 135
patients were culture positive. Similarly, sputum samples from 116 patients were both culture and smear positive. Further, sputum samples from 19 patients were smear negative but culture positive. In addition, samples from five patients were both culture and smear negative. Sputum samples from 13 patients were contaminated during culture.

Case-wise distribution of PTB patients
Out of 135 Mycobacterium tuberculosis isolates, 31.1 % (n = 42) isolates were isolated from new TB cases and 68.9 % (n = 93) isolates were isolated from the previously treated TB cases. Among previously treated TB cases the highest number of cases were relapse (59.1 %) followed by treatment failure cases (15.1 %), chronic cases (11.8 %), return after default cases (8.6 %) and follow-up cases (5.4 %).

Drug susceptibility patterns of Mycobacterium tuberculosis
Of total 135 Mycobacterium tuberculosis isolates, 68.9 % (n = 93) were sensitive to all four drugs and 31.1 % (n = 42) were resistant to any drugs. The highest rate of resistance was toward streptomycin (SM or S) (24.4 %) followed by isoniazid (INH or H) (23 %), rifampicin (RMP or R) (17.8 %) and ethambutol (EMB or E) (15.6 %). Among the total of 135 culture positive TB cases, 21(15.6 %) were found to be MDR-TB. The prevalence of MDR-TB (19.4 %) among the previously treated cases was significantly higher than that among newly diagnosed cases (7.1 %) and the difference was statistically significant. Around 6.7 % of the total culture positive TB cases were poly-resistant TB (resistant to more than one drug but not MDR-TB). No poly-resistant TB cases were reported among newly diagnosed tuberculosis patients (Table 1).

Drug susceptibility patterns of Mycobacterium tuberculosis isolated from different cases of previous treated patients
Among the total of MDR-TB cases among previously treated patients, highest percentage of the cases were relapse (61.1 %) followed by chronic cases (16.7 %), treatment failure cases (11.1 %), return after default cases (5.6 %) and follow-up cases (5.6 %). Drug susceptibility patterns of Mycobacterium tuberculosis isolated from different groups of previously treated patients are presented in Table 2.

Discussion
Similar to our finding, Hajoj et al. in Saudi Arabia reported the prevalence of MDR-TB among previously treated TB patients to be 15.9 %, however they showed lower rate of MDR-TB (in comparison to our study) among newly diagnosed cases (1.8 %) [15]. Similarly, Rijal et al. in their study during 2003 to 2004 found the rates of MDR-TB among previous treated and new cases to be 19.3 % and 2.6 % respectively [16]. However, Subba et al. reported quite high rate of MDR-TB cases (i.e. 22.2 % among new cases and 37.2 % among previously treated cases) in a study during 2005 to 2006 [17]. Further, during 2006 to 2007 Pradhan et al. discovered the prevalence of MDR-TB to be 15.5 % among previously treated cases and 4.2 % among new cases [18]. The most recent drug resistance surveillance of 2011 in Nepal, reported the prevalence of MDR-TB to be 2.6 and 17.6 % among new and previously treated cases respectively [6]. Another study during 2011 to 2012 by Tharu et al. noted the rate of MDR-TB among culture positive retreatment cases to be 33 % [19]. A study conducted by Khunjeli et al. in Nepal from 2012 to 2014 discovered the prevalence of primary drug resistant tuberculosis to be 4.8 % [20]. The drug susceptibility patterns of M. tuberculosis isolated in different studies conducted in Nepal during different periods of time have been presented in Table 3 [16–18, 21]. Over the years, there has been gradual increase in primary MDR-TB in Nepal. This may be attributed to the increased rate of the acquired resistance, which contributes to the rise in primary drug resistant TB [6]. The main causes of high rates of drug resistance among previously treated tuberculosis cases in Nepal may be improper and/or incomplete treatment [22].

| Susceptibility patterns | Total cases (N = 135) n (%) | New cases (N = 42) n (%) | Previously treated cases (N = 93) n (%) |
|-------------------------|----------------------------|--------------------------|---------------------------------------|
| Any drug resistance     | 42 (31.1)                  | 9 (21.4)                 | 33 (35.5)                             |
| Any isoniazid resistance| 31 (23)                    | 3 (7.1)                  | 28 (30.1)                             |
| Any rifampicin resistance| 24 (17.8)                 | 4 (9.5)                  | 20 (21.5)                             |
| Mono isoniazid resistance| 2 (1.5)                   | 0                        | 2 (2.2)                               |
| Mono rifampicin resistance| 2 (1.5)                  | 1 (2.4)                  | 1 (1.1)                               |
| MDR-TB                  | 21 (15.6)                  | 3 (7.1)                  | 18 (19.4)                             |
| Poly-resistant TB (resistant to more than one drug, but not MDR-TB) | 9 (6.7) | 0 | 9 (9.7) |
Other risk factors for development of MDR-TB are previous TB treatment, smoking habit, poverty, illiteracy, migration to/from high MDR-TB prevalence areas, alcoholism, poor housing, overcrowding and homelessness [23, 24]. The mode of transmission of drug-resistant TB is same as that of drug susceptible TB. And the transmission of the MDR-TB is a very serious public health issue, as it is very difficult to treat MDR-TB.

In accordance to our finding, in a study by Massi et al., 60.9 % of the total M. tuberculosis isolates were found to be sensitive to all four drugs, while 39.1 % of the isolates were resistant to at least one of the four drugs [25]. However, Pradhan et al. [18] and Rijal et al. [16] noticed the rates of any drug resistance to be 52.5 and 67.8 % respectively.

Higher rate of sensitivity to RMP alone is a good indicator for success of directly observed treatment short course (DOTS) program but in our study 9.5 % of the isolates were resistant to at least one of the four drugs [25]. Under DOTS program, early screening and treatment in MDR-TB cases. Early diagnosis, good quality treatment and follow up cases are the patients who are called in follow up even after successful completion of anti-tubercular therapy, chronic cases are the patients who have received repeated incomplete treatment and who still have active tuberculosis that is considered to be incurable [6].

Findings of our study suggest that relapse cases are predominant over the other previously treated tuberculosis cases. Similar to our study, Al-Marri (85 %) in the state of Qatar [28] and Subba et al. (68.4 %) in Nepal [17] reported that the highest number of cases in previously treated TB patients were relapse.

The presence of DR/MDR-TB reflects poor tuberculosis control in the present or past which creates great problem to effective implementation of TB control program throughout the world [15]. So, for the effective implementation of the TB control program, it is necessary to have the laboratories where MDR-TB cases can be detected but they are not sufficiently available in developing countries like Nepal [6]. In case of under developing countries like Nepal, the rapid GeneXpert MTB/RIF assay may be proved to be promising for detection of MDR-TB cases. Early diagnosis, good quality treatment under DOTS program, early screening and treatment in closed contact and infection control program may be very useful to improve health care service, hence to control the MDR-TB.

Table 2 Drug susceptibility patterns of Mycobacterium tuberculosis isolated from different groups of previously treated patients

| Susceptibility patterns | Total cases (N = 93) n (%) | Relapse (N = 55) n (%) | Treatment failure (N = 14) n (%) | Return after default (N = 8) n (%) | Follow up (N = 5) n (%) | Chronic (N = 11) n (%) |
|------------------------|---------------------------|------------------------|-------------------------------|---------------------------------|------------------------|-----------------------|
| Any drug resistance    | 33 (35.5)                 | 18 (32.7)              | 4 (28.6)                      | 4 (50)                          | 1 (20)                 | 6 (54.6)              |
| Any isoniazid resistance | 28 (30.1)                | 16 (29.1)              | 2 (14.3)                      | 3 (38)                          | 1 (20)                 | 6 (54.6)              |
| Any rifampicin resistance | 20 (21.5)                | 11 (20)                | 3 (21.4)                      | 2 (25)                          | 1 (20)                 | 3 (27.3)              |
| Mono isoniazid resistance | 2 (2.2)                  | 1 (1.8)                | 0                             | 0                              | 0                      | 1 (9.1)               |
| Mono rifampicin resistance | 1 (1.1)                  | 0                      | 1 (7.1)                       | 0                              | 0                      | 0                     |
| MDR-TB                 | 18 (19.4)                 | 11 (20)                | 2 (14.3)                      | 1 (12.5)                       | 1 (20)                 | 3 (27.3)              |
| Poly-resistant TB (resistant to more than one drug, but not MDR-TB) | 9 (9.7)                | 4 (7.3)                | 0                             | 4 (50)                          | 0                      | 2 (18.2)              |

Note: Relapse cases are the patients who become symptomatic after completion of the treatment, treatment failure cases are the patients who remain smear positive or culture positive even after 5 or more months of start of anti-tubercular therapy, default cases are the patients whose treatment is halted for at least two consecutive months, follow up cases are the patients who are called in follow up even after successful completion of anti-tubercular therapy, chronic cases are the patients who have received repeated incomplete treatment and who still have active tuberculosis that is considered to be incurable [6].
### Table 3: The drug susceptibility patterns of *M. tuberculosis* isolated in different studies conducted in Nepal during different periods of time

|                            | National survey 1996–1997 [21] | National survey 1998–1999 [21] | National survey 2001–2002 [21] | Rijal et al. 2003–2004 [16] | Subba et al. 2005–2006 [17] | Pradhan et al. 2006–2007 [18] | National survey 2006–2007 [21] | Finding of our study 2013 |
|---------------------------|--------------------------------|--------------------------------|--------------------------------|-----------------------------|------------------------------|------------------------------|--------------------------------|-------------------------|
|                           | New cases | Previously treated cases | New cases | Previously treated cases | New cases | Previously treated cases | New cases | Previously treated cases | New cases | Previously treated cases | New cases | Previously treated cases | New cases | Previously treated cases | New cases | Previously treated cases |
| Any drug resistance       | 9.8 %      | 0                              | 13.2 %     | 28.6 %                     | 11.0 %     | 40.9 %                     | 36.8 %     | 75.2 %                     | 64.5 %     | 74.8 %                     | 46.5 %     | 67.2 %                     | 4.7 %       | 25.3 %                     | 21.4 %     | 35.5 %                     |
| Mono-drug resistance      | 5.7 %      | 0                              | 7.6 %      | 11.6 %                     | 7.0 %      | 13.0 %                     | 23.7 %     | 32.3 %                     | 20 %       | 23.6 %                     | 24.3 %     | 25.9 %                     | 9.1 %       | 6.1 %                      | 14.3 %     | 6.5 %                      |
| Multidrug resistance      | 1.1 %      | 0                              | 3.7 %      | 12.5 %                     | 1.32 %     | 20.5 %                     | 2.6 %      | 19.3 %                     | 22.2 %     | 37.2 %                     | 4.2 %      | 15.5 %                     | 2.9 %       | 11.7 %                     | 7.1 %      | 19.4 %                     |
| Resistance to all 4 first line drugs | 0          | 0                              | 1.8 %      | 9.8 %                      | 0.8 %      | 9.4 %                      | 2.6 %      | 11.8 %                     | 6.7 %      | 18.8 %                     | 0          | 6.9 %                      | 1.8 %       | 6.8 %                      | 4.8 %      | 15.1 %                     |
DOTS can treat virtually all the patients with drug susceptible TB [29]. And for the patients with MDR-TB, DOTS Plus can be used for the treatment and to prevent the development of the further drug resistance [30]. But under DOTS Plus second-line injectable drugs, which are inherently more toxic and less effective than first-line drugs, are used daily for 2 years [30]. The treatment is more expensive and prolonged. So it is necessary to confirm MDR-TB (before starting treatment) in case of failure of DOTS rather than presuming the patients failing DOTS to have MDR-TB and starting the 2nd line anti-tubercular therapy [30].

Limitations of the study
Due to lack of resources, we could not include more samples in our study. Multi-center studies including large number of samples could have generated more significant data. We could not use molecular methods for the identification of the Mycobacterium tuberculosis.

Conclusions
In our study high rate of multidrug resistance was noted. On the basis of the drug susceptibility patterns of M. tuberculosis we found in our study, we recommend to include ethambutol instead of streptomycin in the multidrug therapy for the treatment of tuberculosis patients in Nepal. Further, due to high rate of MDR-TB among previously treated patients, we do not recommend to use first line drugs for the treatment of pulmonary tuberculosis among previously treated patients. The empiric treatment of tuberculosis should be based on the local drug susceptibility patterns of M. tuberculosis. Rapid testing for drug resistance by some methods like GeneXpert MTB/RIF assay and improvement of infection control program may be helpful in control of the tuberculosis.

Abbreviations
AIDS, acquired immune deficiency syndrome; DOTS, directly observed treatment short course; DR-TB, drug resistant tuberculosis; DST, drug susceptibility testing; EMB or E, ethambutol; GENETUP, German-Nepal tuberculosis project; HIV, human immunodeficiency virus; INH or H, isoniazid; L-L, Lowenstein-Jensen medium; MDR-TB, multidrug-resistant tuberculosis; PTB, pulmonary tuberculosis; RMP or R, rifampicin; SM or S, streptomycin; SPSS, statistical package for the social sciences; TB, tuberculosis; WHO, world health organisation

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Availability of data and materials
The raw data can be made available to the interested researchers by the authors of this article if requested.

Authors’ contributions
NDP designed and carried out the research works, analyzed data, and prepared the manuscript. GT designed and carried out the research works, analyzed data. SK contributed in analysis of the data. BL and BS monitored the study. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Before start of the research, the protocol was approved by German-Nepal Tuberculosis Project Laboratory, Kathmandu, Nepal and Goldengate International College, Kathmandu, Nepal. Informed consent was obtained from all the patients or patient’s guardians. The research was in compliance with the Helsinki Declaration.

Author details
1Department of Microbiology, Goldengate International College, Battisputali, Kathmandu, Nepal. 2Department of Microbiology, Grande International Hospital, Dhapasi, Kathmandu, Nepal. 3Department of biochemistry, Modern Technical College, Lalitpur, Kathmandu, Nepal. 4German-Nepal Tuberculosis Project (GENETUP), Kalimati, Kathmandu, Nepal.

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