Original Research Paper

Study of Anti-Tuberculosis Drug Resistant Pattern among the Tuberculosis Patients Visiting German Nepal Tuberculosis Project

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Accepted 16th September, 2016.

Tuberculosis (TB) is a major public health problem in Nepal. Multidrug-resistant (MDR) strains are serious threats to the control of TB and comprise an increasing public health problem. The aim of this study was to determine the anti-tuberculosis drug resistance pattern among TB patients visiting German Nepal Tuberculosis Project (GENETUP) by using proportion method. The descriptive cross-sectional study was carried from November 2010 to June 2011. During the study period, 112 pulmonary tuberculosis (PTB) patients attending GENETUP were included in the study. Of a total of 112 culture positive isolates, 96 were from re-treatment cases and 16 were from new cases. Among the culture positive cases, the highest number of culture positive cases belonged to the age group of 21-30 years (36.7%) followed by 11-20 years (14.3%). Out of total tested cases, the patients sensitive to all four drugs were found to be 37.5% (n=42). The patients that showed resistance to only one drug was found to be 3.6% (n=4) and resistance to two drugs 26.8% (n=30). Similarly, resistance to three drugs was found to be 22.4% (n=25) and resistance to all four drugs was 9.9% (n=11) among both cases. Regular monitoring of acquired MDR-TB patients and revising anti-tuberculosis treatment policy should be made at the national level representative for controlling tuberculosis. This method can be used for the early diagnosis and control of MDR-TB in developing countries including Nepal.

Keywords: Multi-drug resistant, Tuberculosis, GENETUP, Nepal.

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by the bacterium Mycobacterium tuberculosis and characterized by the formation of granuloma in infected tissue as a result of cell-mediated immunity [1]. TB presents a significant health threat to the world’s population, with 9.6 million new cases and 1.5 million deaths per year from this disease [2]. Drug-resistant TB is increasing in many parts of the world and high rates of multidrug-resistant TB (MDR-TB) isolates resistant to at least isoniazid (INH) and rifampin (RMP) have been reported in several countries [3]. Approximately 480,000 new MDR-TB cases are estimated to occur every year [2]. The emergence of drug-resistant Mycobacterium tuberculosis strains has become a global public health problem [4].

The latest (2011/2012) national anti-tuberculosis drug resistance survey conducted in Nepal showed 2.2% MDR-TB in new cases and 15.4% in retreatment cases and among them, 2.4% of TB patients were co-infected with HIV [5]. The worldwide increase in the rates of MDR-TB has made the timely identification of resistant Mycobacterium tuberculosis strains extremely important to achieve effective disease management to prevent their spread. INH and RMP are the most important first-line anti-tuberculosis drugs and resistance to these drugs often results in treatment failures [6]. Treatment for MDR-TB patients requires the use of second-line drugs for more than 24 months. These drugs are more costly, toxic, and less effective than first-line drugs used for routine treatment of TB [7]. As with other diseases, resistant to TB results primarily from lack of adherence to medication by patients, incorrect drug prescribing by providers, poor quality drugs, or erratic supply of drugs [8].

MDR strains are serious threats to the control of tuberculosis and comprise an increasing public health problem.
Patients infected with MDR strains, are difficult to cure and more likely to remain sources of infection for a longer period of time than patients with drug-susceptible strains [9]. In a research performed by Ghimire et al., high rates of MDR-TB isolates were obtained in Nepal. Among the total studied cases, 12.9% isolates were resistant to one or more anti- tuberculosis drug (Rifampicin, Ethambutol, Isoniazid and Streptomycin) with initial and acquired MDR 4.6% and 5% of the isolated respectively [10]. This indicated that utmost rapid detection of the antimicrobial susceptibility pattern in suspected PTB patients is important for the early administration of appropriate therapeutic agents for the prevention of additional resistant development. Therefore, the present study aimed to determine the anti-tuberculosis drug resistance pattern among TB patients visiting GENETUP by using proportion method.

**MATERIALS AND METHODS**

The descriptive cross-sectional study was carried from November 2010 to June 2011. A total of 112 sputum specimens were collected from suspected TB and confirmed MDR-TB patients referred to GENETUP, National Reference Laboratory, Kalimati, Kathmandu, Nepal. Referred sputum samples were processed for smear microscopy, culture and conventional drug susceptibility testing (DST).

**Sample Collection and processing**

During sample collection, three samples were collected first one on the spot, second early morning and the third one again on the spot in which patients were instructed to take a deep breath and cough deeply and vigorously. Patients were instructed to cover their mouths carefully while coughing and not to use oral antiseptics during the period of sample collection. Saliva and nasal secretions were not accepted as described by Forbes et al [11]. All collected sputum specimens were processed for fluorescence microscopy, culture on Lowenstein-Jensen (LJ) media by proportion method which were then analyzed by DST on drug containing LJ media [12].

**Digestion, Decontamination and Concentration**

Digestion, decontamination and concentration were done from per standard methodology [13]. Briefly, 1-5 ml of sample from container was transferred to the 50 ml centrifuge tube with a screw cap using a dropper. Fresh digestant was prepared by breaking ampoule of N-acetyl-L-cysteine (NACL) in BBLM™ Mycoprep™ (specimen digestion/decontamination kits were used for the digestion and decontamination of clinical specimen suspected to contain mycobacteria especially Mycobacterium tuberculosis) NALC-NAOH solution bottle. NALC-NAOH sodium citrate solution (Mycoprep) was added in a volume equal to the quantity of specimen and cap was tightened. The tubes were then vortexed lightly or hand mixed for about 15-30 seconds.

The tube was then inverted to expose the whole solution with NALC-NAOH solution. It was then kept at room temperature, with gentle continuous shaking/rotation for 15 minutes. After completion of shaking, it was left at room temperature for 5 minutes to allow for setting of aerosols. Specimen volume was made up to 50 ml with the addition of sterile Mycoprep phosphate buffer and mixed well. The specimen was centrifuged at 3000 X g for 20 minutes at 4 - 16°C. After centrifugation, the tube was left for 5 minutes to allow aerosols to settle. In the class II biosafety cabinet, decantation was performed for supernatant into a suitable container containing a mycobacterial disinfectant. Using a sterile pipette, pellet was resuspended in 1 ml sterile buffer and lightly Vortexed or mixed with the pipette. The resuspended pellet was then used for LJ culture.

**Sputum samples**

Sputum samples, which were acceptable as per International Union against tuberculosis and lung disease/American thoracic society (IUATLD/ATS) criteria, were further processed for culture [14,15]. All inoculations were done in Biosafety level (BSL) II facility (Laminar flow hood) at GENETUP. 2-3 drops (0.1-0.2ml) of concentrated and decontaminated sputum was inoculated into each of two LJ slants using a sterile dropper.

The slants were laid with the medium face up for 30 minutes to allow the bacteria to adhere to the surface of the medium. The tubes were incubated in an incubator at 37°C for up to 8 weeks and observed any growth every week. If colonies were seen at any stage, acid-fast staining was performed to identify and further processed for culture. Contamination was recorded when it was apparent. Negative report was given when no colonies appeared after observing for 8 weeks. Grading of culture was done and noted as per standard protocol [12].

**Drug Susceptibility Test**

Several 100-fold serial bacilli dilutions were inoculated into drug-containing and drug-free (control) media. The number of colonies obtained in the drug-containing and control media were enumerated. If the proportion of resistant bacteria was higher than 1% for isoniazid, rifampicin and para-aminosalicylic acid or 10% for the other drugs, the strain was considered resistant and the results were taken otherwise, the test was read again at 42 days of incubation to assess if the strain was susceptible to a certain drug [16].

**RESULTS**

**Demographic information of culture positive isolates**

A total number of 112 culture positive isolates, 96 were from retreatment cases and 16 from the new cases. 10.8 % (n=12) male were from new cases, while 54.5 % (n=61) male from retreatment cases. Similarly, 3.6% (n=4) female were from new cases and 31.3% (n=35) female from retreatment cases. The highest number of culture positive cases belonged to the age group 21-30 years (36.6%) followed by 11-20 years (14.3%) as shown in Table 1. Among the MDR patients, males had a higher number than females. This result showed that males were more resistant towards the drugs or antibiotics used for tuberculosis. Males had higher rates of MDR-TB (Table 2).

**Drug Susceptibility test of Retreatment and New cases**

Out of total tested cases, the patients sensitive to all four drugs were found to be 37.5% (n=42). The patients that showed resistance to only one drug were found to be 3.6% (n=4) and resistance to two drugs 26.8% (n=30). Similarly, resistant to three drugs was found to be 22.4 % (n=26) and resistant to all four drugs was 9.9% (n=11) in both the cases as shown in Fig 1. As shown in table 3, among the MDR patients, only 15.2 % were found to be MDR-positive while 84.9% were MDR-negative. Only in the retreatment cases of MDR patients were observed by DST process.
Table 1: Age, sex distribution of culture positive isolates

<table>
<thead>
<tr>
<th>Age group</th>
<th>New patients</th>
<th>Retreatment patients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male No.</td>
<td>%</td>
<td>Female No.</td>
</tr>
<tr>
<td>11-20</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>21-30</td>
<td>7</td>
<td>6.3</td>
<td>3</td>
</tr>
<tr>
<td>31-40</td>
<td>1</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>41-50</td>
<td>1</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>51-60</td>
<td>3</td>
<td>2.7</td>
<td>0</td>
</tr>
<tr>
<td>61-70</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>71-80</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>10.8</td>
<td>4</td>
</tr>
</tbody>
</table>

No: Number of isolates
%: Percentage of isolates

Table 2: Gender wise distribution of MDR cases

<table>
<thead>
<tr>
<th>Sex of MDR Patients</th>
<th>No. of Patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10</td>
<td>58.9</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>41.1</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 1: Pattern of Drug Susceptibility test of Retreatment and New cases
DISCUSSION

Development of drug resistance in *Mycobacterium tuberculosis* isolates due to random genetic mutations in particular genes conferring for resistance. Among the countries listed in the WHO report India, and China had the highest burden of MDR-TB together accounting for almost half of the world total cases [17]. According to national drug resistance survey in 2006, the prevalence of MDR-TB in Nepal among the new and retreatment cases were 2.95 and 11.7% respectively [18]. In the present study, drug susceptibility test out of 112 (both new and retreatment cases) 17 (15.2%) cases were found to be multi-drug resistant (resistant to isoniazid and rifampicin). The trends of MDR-TB cases were increasing due to the sharing of open border within the neighboring countries India. Patients from north India come to Nepal for cheaper treatment facilities so that frequent airborne transmission occurred among the patients [19].

In this study, 1(15.2%) MDR isolated from both retreatment and new cases out of 112 positive culture isolates. These results show that the lower rate of MDR than the other previous study carried by (Khati BB, 2012) [20], follow up retreatment MDR positive patients were included in their research study. The prevalence of tuberculosis was found higher in male than female as male being more exposed towards the outer environment. TB patients who do not take standard regimen treatment including an appropriate combination of anti-TB drugs (Isoniazid, Rifampicin, Pyrazinamide and either Ethambutol or Streptomycin) for required length of time and they usually develop drug-resistant tuberculosis.

The maximum number of suspected cases as well as positive cases were from age group 21-30 years (36.7%) followed by 11-20 years (14.3%). Statistically, there was no significant difference between gender wise distribution of culture positive between new and retreatment patients ($\chi^2 = 4.800$, $p = 0.308$, $\chi^2 = 11.698$, $p = 0.069$), which is not in agreement with previous studies [21,22]. The reason may be that the middle age group (21-30) years is more exposed to the outdoor activity in search of jobs and other activities like business, trade, study, pilgrimage, cultural visits to our near neighbor country India so that there is a high risk of TB transmission with this age group. Since drug-resistant rates on one side of the border impact the other side of the border, a high proportion of MDR-TB in Nepal may reflect the possible dissemination of infection from surrounding two countries, mainly from India [19].

Among the 112 culture positive isolates obtained from the previously treated PTB patients, 96 isolates from retreatment cases (include relapse, chronic, follow up, defaulter, and treatment failure) and 16 isolates from new cases which were not previously treated for tuberculosis. The patients sensitive to all four drugs found to be 37.5% (n=42). The patients that showed resistance to only one drug was found to be 3.6% (n=4) and resistant to two drugs 26.8% (n=30). Similarly, resistance to three drugs was found to be 22.4% (n=25) and resistance to all four drugs was 9.9% (n=11) in all cases. Resistance to all four drugs was almost absent in new cases. This finding is not in accordance with previous studies because the Genotype MTBDR plus assay method have more sensitivity and specificity comparing than the conventional DST method [21,24]. It may be due to the reason that they are just in the initial stage of infection which could be cured by proper treatment of tuberculosis.

In a research study performed by Dahal et al., [24] low rates of MDR-TB cases were isolated as compared this study result obtained. MDR-TB is increasing due to the lack of treatment compliance, supply of poor quality drugs and recent transmission from MDR patients. Hence, MDR-TB has become an issue of increasing importance in both developed and developing countries [25]. In Bangladesh, MDR-TB increases from 2% to 5.5% with the progress of time [26]. The increasing spread of MDR, reemergence of Extensively drug-resistant TB (XDR-TB) with additional resistance to fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin) pose a significant threat to tuberculosis control [18].

This study was done in the retreatment and new cases referred to the National tuberculosis center Kathmandu from the different part of the country. The patients who referred were relapse, chronic, treatment interrupted; follow-up, treatment failure as well as the serious patients for admission. Therefore, the studies conducted at GENETUP may have a higher prevalence than in the community-based health care centre.

CONCLUSION

Regular monitoring of acquired MDR-TB patients and revising anti-tuberculosis treatment policy should be made by the national level representative for controlling tuberculosis. The high percentage of MDR may be considered as an indication for starting the programme like DOTS strategy. Hence, this method can be used for the early diagnosis and control of MDR-TB in developing countries including Nepal.

CONFLICT OF INTERESTS

None

ACKNOWLEDGEMENT

We would like to thank the team of German-Nepal Tuberculosis Project (GENETUP), Kathmandu, Nepal for providing laboratory facilities, required reagents and help during research work.

Table 3: Pattern of MDR cases

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Particular</th>
<th>Retreatment Cases</th>
<th>New Cases</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MDR INH + RMP</td>
<td>16</td>
<td>1</td>
<td>17</td>
<td>15.2</td>
</tr>
<tr>
<td>2</td>
<td>Non-MDR</td>
<td>80</td>
<td>15</td>
<td>95</td>
<td>84.9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>16</td>
<td>112</td>
<td>100</td>
</tr>
</tbody>
</table>

INH: Isoniazid
RMP: Rifampicin

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