ABSTRACT

Tuberculosis is an infectious disease and it is one of the leading causes of death worldwide especially the third world countries. With the emergence of multi drug resistant strains, the fatality rate is continuously increasing (WHO, 2009). Early diagnosis of multi drug resistant tuberculosis is crucial for better treatment and public health control. India alone accounted for an estimated 26 percent of all TB cases worldwide, and China and India combined accounted for 38 percent. TB remains a major killer in India, killing two persons every three minutes, nearly 1,000 a day (WHO, 2011).

A total of 375 sputum specimens (smear positive) of clinical isolates collected from Northeast India were investigated. The primary aim of this study is to analyse the drug susceptibility of *Mycobacterium tuberculosis* of the collected samples. Conventional and rapid methods of drug susceptibility tests were done on the sputum samples. Drug susceptibility testing was performed for 375 smear positive isolates by proportion method (PM), microscopic observation drug susceptibility (MODS), Nitrate Reductase Assay (NRA), BacT/Alert and Genotype MTBDR methods. Comparisons of results obtained by different methods were further investigated in order to highlight the efficient and rapid methods for TB detection and analysis of drug resistant.

The rate of relapse of previously treated cases of TB was found to be high in the male population with 31.2% in comparison to female population 14% and children 2.4%. Of the total sample, isolates showing resistance to RIH (rifampicin), INH (isoniazid), STR (streptomycin) and EMB (ethambutol) were 28% (n = 105/375), 31.4%, 20.8% and 28% respectively by proportion method. 47 samples were found MDR positive by proportion method.

MODS detected isoniazid (INH), rifampicin (RIH), streptomycin (STR) and ethambutol (EMB) resistant samples at 31%, 29%, 19.3% and 27.3% respectively and 51 samples as MDR positive.

A comparison of the sensitivity of the four drugs in MODS shows a relatively low percentage in STR (93.59%) with high value in RIH (100%). Specificity was very high for all the drugs resistance with more than 98%. MODS method of DST is found
to be comparatively rapid and cost efficient and very much suitable method in resource-limited regions like Northeast India.

In NRA method, 30.1% of samples were detected as rifampicin resistant, 32.8% as isoniazid resistant which is highest among the four drugs. For streptomycin resistant 18.6% of samples was detected which is lowest among four drugs while 31.4% of samples were detected as ethambutol resistant and 48 samples were detected as MDR positive. The results of DST (drug susceptibility test) by NRA showed that isoniazid (INH), rifampicin (RIH) and ethambutol (EMB) had sensitivity at 100% each, while it was marginally low for streptomycin (STR) at 89.74%.

Bact/Alert 3D method detected 60 samples as MDR positive strain, 15 samples detected culture negative while 20 samples tested mono resistant to rifampicin and 16 samples tested mono resistant to isoniazid.

By Genotype MBDRplus, 58 samples were detected as MDR positive, 21 samples positive for mono-resistant to rifampicin while 16 samples mono-resistant to isoniazid. Specificity of 100% was seen in both rifampicin and MDR while 99.21%. The RIH resistant isolates displayed different mutations. The most common mutation was in the S531L region (MUT3) with 45.6% of all RIH (Rifampicin) resistant strains. 3.4% of MDR had a mutation in the S315T2 region indicating high level resistance of isoniazid.

All MDR (Multi Drug Resistant) samples tested for XDR by MTBDRsl were fortunately negative which indicates that XDR is not yet detected from this region during the study. One MDR positive strain had a gyrA MUT3D mutation at D94H region. This is a rare mutation, which has been detected only in silico and hence thought to be undetectable in vitro. However, this detection albeit in one strip proves that it is detectable and that the MTBDRsl is efficient in detecting even rare mutations.

Regardless of the choice of restriction enzyme, reproducible results were obtained with all mycobacterial species tested for ARDRA. The cluster analysis of the ARDRA profiles showed grouping of the MTBC species into one clade with *M. avium* as its sister species. This is also supported by the 16S rRNA phylogram. Amongst all these species, *M. flavescens* found to be the most diverged from the rest of the species.