Predominance of *Mycobacterium tuberculosis* Beijing genotype among presumptive multidrug resistance patients from North East India

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Northeast India is the easternmost region of India and comprises of Seven Sister States—Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura—and the Himalayan state of Sikkim. Though there are many studies characterizing M. tuberculosis isolates from different parts of India, studies highlighting genotypic diversity among isolates from north east India are rare. The present study aimed to obtain an initial insight into the predominant spoligotypes prevalent in north east region. Sputum samples from presumptive MDR patients were received from the north east states for Drug suseptitibility testing (DST). All specimens were screened for presence of AFB by Ziehl-Neelsen (ZN) staining. The samples were processed by N-acetyl-L cysteine - Sodium hydroxide (NALC-NaOH) method. Drug susceptibility testing (DST) was performed by either Line Probe Assay (Hains, MTBDR plus for RIF and INH) or by MGIT960 liquid culture system (Becton Dickinson, Sparks, MD) after routine mycobacterial identification. (RIF; 1 µg/ml, INH; 0.1 µg/ml). 148 isolates randomly selected from existing stocks were subjected to spoligotyping as per standard protocol and spoligo patterns obtained were compared to those within the SITVIT2 database. The age of the patients varied from 10-77 years with mean age of 31 years. 99 (67.0%) patients were male and 49 (33%) were female with a male female ratio of 2: 1. 28 (19%) isolates were sensitive to both the drugs whereas 107 (72.3%) isolates were MDR. 8(5.4%) isolates were INH resistant and 2 (1.3%) were mono RIF resistant. Spoligotyping yielded 31 different patterns, 23 of these were unique (1 isolate only) whereas 8 patterns containing 125 isolates were clustered (2 or more isolates) with a clustering rate of 84.45%. SIT 1 predominated in this study with 104/148 (70.2%) isolates. Apart from SIT 1 there were no major clusters. The predominant family in this study was found to be Beijing (73.64% of total strains). Other families corresponded to Central Asian (CAS) and East African Indian (EAI). The Harlem family, the poorly defined T family and "Manu" were present as minor families. Among 107 MDR strains, 85 belonged to SIT1. Of these mutation data was available for 67 isolates. For rifampicin, commonest mutation was at codon S531L of rpoB gene (58/67; 86.5%) followed by H526Y (2/67; 3.0%) and D516V (2/67; 3.0%). In 5 isolates (7.5%), resistance was determined by absence of wild type probes alone. For INH high level resistance corresponding to mutation in codon 315 of Kat G gene occurred in 97.0% (65/67) of samples whereas low level resistance in -15 promoter region was present in two strain. According to previous available data EAI and CAS were found to be predominant in India. High percentage of Beijing genotype in north east India is significantly higher than the rest of India. Considering the fact that this genotype is the major cause of outbreaks involving drug resistant variants worldwide, our findings are major cause of concern for health authorities.

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