[1]*Abdolrazagh Hashemi-Shahraki,* [2]*Saeed Zaker Bostanabad,* [3]*Titov L. P.*

SPECIES SPECTRUM OF NONTUBERCULOUS MYCOBACTERIA ISOLATED FROM SUSPECTED TUBERCULOSIS PATIENTS, IDENTIFICATION BY MULTI LOCUS SEQUENCE ANALYSIS

[1] *Infectious and Tropical Disease Research Center, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran*
[2] *Microbiology Department, Islamic Azad University-Parand Branch, Tehran, Iran*
[3] *The Belarusian Scientific-Research Institute of Epidemiology and Microbiology, Minsk*

Identification of Mycobacterium species is difficult due to a complex and rapidly changing taxonomy, the failure of 16S rRNA to discriminate many closely related species and the unreliability of phenotypic testing. We investi-

gated a collection of nontuberculous mycobacteria (NTM) strains isolated from suspected tuberculosis patients at Tuberculosis Reference Centre (Ahvaz, Iran) and Masoud Laboratory (Tehran, Iran) during 2008–2012 to evaluate the species spectrum of NTM isolates. Based on phenotypic tests, the isolates were identified up to species or complex level; however they were heterogonous by hsp65-PCR restriction fragment length polymorphism analysis (PRA) method.

Representative isolates from each hsp65-PRA pattern, were subjected to identification using single locus and multi locus sequence analysis (MLSA) based on 16S rRNA, rpoB, hsp65 and 16S–23S internal transcribes spacer (ITS) fragments to determine their taxonomic affiliations. All 92 NTM isolates from different clinical specimens were considered as etiological agents causing disease according to American Thoracic Society (ATS) guideline. Phenotypic evaluation alone assigned 66 (72 %) isolates to a species or complex level and consequently 76 (82 %) isolates showed previously reported hsp65-PRA patterns. Although sequence base identification using single locus such as 16S rRNA, rpoB, hsp65 or ITS identified the isolates up to species level, MLSA correctly identified 16 different species of NTM from clinical isolates.

In summary, four-locus MLSA is a reliable method for elucidating taxonomic data and reliable species identification of Mycobacterium isolates and therefore, would be more feasible for routine use in Tuberculosis (TB) reference laboratory.