ABSTRACT

Sequencing vs MALDI TOF- Tools for Accurate Identification of Non-Tuberculous Mycobacteria- a Pilot study

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Introduction:

Non-Tuberculous Mycobacteria (NTM) cause various opportunistic diseases in humans. Accurate identification of NTMs up to species level is not only important to know the clinical significance of these organisms but also for appropriate management as antimicrobial treatment is species specific.

Objectives

The objectives of the study were to standardize the 16srRNA and ITS methods of sequencing of Non-Tuberculous Mycobacteria (NTM) and evaluate the Matrix Assisted Laser Desorption/Ionization- Time of Flight (MALDI- TOF) technology against the gold standard sequencing methods for accurate speciation of NTMs.

Materials and methods

Ninety NTM strains isolated from various clinical samples at the Department of Clinical Microbiology at Christian Medical College, Vellore were included. Conventional biochemical tests were done as per the standard protocol. We performed sequencing of the 16S rRNA and MALDI-TOF mass spectrophotometry on all 90 NTM isolates. In addition ITS sequencing was performed on the slow growers. Clinical details of patients from whom these isolates were cultured was obtained from electronic patient records. The results were entered into Epidata software ver 3.1. Analysis was done using SPSS 16.0 and Microsoft Excel.

Results

In this study,49/90 (54%) were slow growers and 41 (46%) were rapid growers. Based on 16S rRNA sequencing, which is the gold standard, they were identified up to the species level.

Most common species were *Mycobacterium abscessus, Mycobacterium intracellulare, Mycobacterium fortuitum, Mycobacterium simiae* and *Mycobacterium avium.* The most common clinical form of disease caused by slowly growing NTM was pulmonary disease (40/49= 81.6%). The predominant clinical manifestation due to rapidly growing NTMs were skin and soft tissue infections (16/41 =39%). The cumulative concordance of MALDI-TOF with the gold standard 16S rRNA sequencing was 83.33% (95% CI: 63.9-81.4). Concordance of MALDI-TOF for the rapid growers with 16S rRNA sequencing was 94.5% (95% CI: 81.3- 99.4) and for slow growers it was 77.5% (95% CI: 63.9-

81.4). The concordance of MALDI-TOF with ITS sequencing (for slow growers) was found to be 76% (95% CI: 63.9- 81.4). Sequencing is 5 to 6 times more expensive than MALDI-TOF and therefore should be used as a confirmatory test. MALDI-TOF requires only protein extraction, which is a simple technique which can be performed by any laboratory technologist in a routine clinical microbiology laboratory setting. MALDI-TOF is a rapid method which will take only 1.5 to 2 hours for the whole procedure, whereas sanger sequencing will take on average 12- 24 hours of turnaround time.

Conclusion

MALDI-TOF assay can be used as a rapid and cost-effective method for identification of NTM in a routine diagnostic laboratory especially for the rapidly growing NTMs. However, for the slow growing and novel or rare NTMs additional sequencing of 16s rRNA and ITS region need to be done for accurate identification.

Keywords: Non-tuberculous mycobacteria (NTM), Matrix Assisted Laser Desorption/Ionization- Time of Flight (MALDI-TOF), 16S rRNA sequencing, ITS sequencing