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Paper Poster Session
Tuberculosis - diagnosis and drug resistance

Molecular identification of non-tuberculous mycobacteria: detection of rare and unidentifiable species

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Background: Non-tuberculous Mycobacteria (NTM) are environmental bacteria that cause opportunistic infections in humans. At present, more than 160 distinct species have been validly published. The introduction of molecular techniques has facilitated the identification NTM species, the clinical relevance and the optimal treatment regimens of which differs strongly by species.We describe our experience regarding the molecular identification of NTM species recovered from clinical specimens in Crete, with emphasis on detection of rare and unidentifiable NTM (UNM), by using more advantaged molecular identification methods as sequence analysis.

Material/methods: Specimens submitted for mycobacterial culture between 1/2000-12/2013 and the recovered NTM were identified by the commercially available DNA strip test Genotype Mycobacterium CM and AS (Hain-Lifescience), two reverse hybridization-based assays. Sequencing analysis of 16S *rDNA* (1500bp) and *hsp65* (440bp) genes was performed when necessary. Sequences were compared with those of validly published species available in the GenBank database (http://www.ncbi.nlm.nih.gov).

Results: In total, 300 NTM isolates from different patients were recovered, 285 of them belonged to 34 known *Mycobacterium* species. The 76.3% (229) of strains (16 species) were correctly identified at the species level by the commercial assays. The most frequent were: *Mycobacterium gordonae* (n=66), *M.lentiflavum* (n=43) and *M.avium* (n=33). The 6% (18) of strains belonged to the recently described species M.*marseillense, M.timonense*, *M. chimaera*, *M.massilliense, M.bolletii*, *M.parascrofulaceum and M.kumamotonense* and were incorrectly identified by commercial assays probably due to the cross-hybridization of such novel species with some of the probes. The 14.7% (38) of strains which were identified by the commercial assays only to the species level, belonged to rare NTM species *M.thermoresistibile, M.rhodesiae*, *M.bohemicum*, *M.neoaurum*, *M.arupense, M.elephantis, M.monacense* and to the recently described (after 2011) species *M.heraklionense, M.europaeum, M.iranicum*, *M.sinense* and *M.celeriflavum*. Finally 15 strains (5%), belongs to 11 species, had unique *hsp65* and 16S *rDNA* sequences (UNM). Three species were slowly (SGM) and 8 were rapid growers (RGM). According to 16S *rDNA* and *hsp65* sequences two SGM belonged to *M.terrae complex* one to *M.avium complex* and the 8 RGM species were closely related to NTM

species *M. monacense*, *M. vaccae*, *M.manitobense*, *M.elephantis*, *M.rutilum*, *M.neoaurum*, *M.iranicum* and *M.duvalii*, respectively, to which has the highest percentage of similarity (16S *rDNA*: <99.6%, *hsp65*: <96%). Phylogenetic analysis of 16S *rDNA* and *hsp65* sequences of UNM, demonstrates that they didn't belong to a previously reported species and represented probably novel *Mycobacterium* species which will be documented with further genomic studies.

Conclusions: Our findings suggest that the combined use of molecular commercial identification tests with sequencing analysis improve the ability to correctly identify not only the common NTM but also the rare, the recently described as well as to detect probably the novel mycobacterial species, leading to better assessment of clinical relevance and to optimal treatment.