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**Objectives.** Nontuberculous 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 of NTM species, the clinical relevance and the optimal treatment regimens of which differs strongly by species. We describe the experience for the molecular identification of NTM species recovered from clinical specimens in Crete, Greece during the decade 1/2001-12/2010.

**Materials and Methods.** Clinical samples (n=12450) submitted for mycobacterial culture were processed and cultured by standard methodology. NTM were identified with the commercial Genotype Mycobacterium CM and AS assays (HainLifescience). Sequencing analysis of 16S rDNA (1500 bp), and of 440 bp fragment of the 65-kDa heat shock protein (hsp65) genes was performed when necessary. Sequences were compared with those of validly published species available in the GeneBank database.

**Results.** During the study period, 291 NTM isolates were recovered from 209 patients, which belonged to 28 species. Only 14 species (169 strains) were correctly identified by Genotype CMAS. The most frequent were: *Mycobacterium gordonae* (n=49), *M. lentiflavum* (n=41), *M. avium* (n=21), *M. fortuitum* (n=17) and *M. kansasii* (n=10). On the other hand, sequencing analysis revealed that Genotype CMAS erroneously identified the recently described species *M. marseillense* (n=7), *M. parascrofulaceum* (n=2), and *M. kumamotoense* (n=1) as *M. intracellulare*, *M. scrofulaceum*, and *M. celatum* respectively and *M. massiliense* (n=1) and *M. bollettii* (n=1) as *M. abscessus*. Finally, 29 NTM strains were initially identified by the Genotype CMAS assay only to the species level. Sequencing analysis indicated that 14 of them belongs to 5 rare NTM species: *M. arupense* (n=8), *M. elephantis* (n=2), *M. monacense* (n=2), *M. thermoresistibile* (n=1) and *M. bohemicum* (n=1). For the other 15 isolates we found four different 16S rDNA and hsp65 gene sequences not matching, during the study period, with sequences of validly published species available in the GeneBank database. Twelve isolates which were recovered from different body sites, had identical sequences, and were closely related to *M. terrae*. These strains belonged to the recently described (2013) species *M. heraklionense*. One strain isolated from sputum with 16S rDNA sequence identical to sequevar MCRO19. This strain belonged to the recently described (2011) species *M. europaeum*. Another one strain isolated from CSF, belonged to the recently described (2013) species *M. iranicum*. Finally a strain isolated from sputum belongs to *M. avium complex* and represent a unique taxonomic entry. We propose for this strain the name '*M. hellenicum*'. The type strain is NCTC 13432 = LGM 24834 = CECT 7461.

**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 and novel mycobacterial species, leading to better assessment of clinical relevance and to optimal treatment.