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Paper Poster Session
Non-tuberculous mycobacteria

Identification of non-tuberculous mycobacteria by using commercial DNA probes and gene sequencing: nine-year experience of a single center in Italy

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Background: The diagnosis of NonTuberculous Mycobacteria (NTM) infections frequently runs into difficulties in the correct species identification. The commercial molecular systems are the most frequently used methods for the identification of NTM isolated by culture, recognizing only a limited number of species and giving several cross-reactivities. In undetermined or ambiguous cases, the NTM identification should be done by the gene sequencing considered the reference method. The aim of this study was to evaluate how many types and the frequency of NTM species were detected in nine-year experience by using a commercial method supplemented with automated Sanger sequencing.

Material/methods: We analyzed 1037 strains of NTM isolated from clinical specimens of 832 patients. The analyzed strains were isolated mainly from respiratory samples. All strains were analyzed for species identification by using GenoType Mycobacteria CM/AS kit (Hain, Lifescience, Nehren, Germany). The unidentified strains were then analyzed by Sanger sequencing carried out on full 16S rDNA and in some cases also on partial rpoB gene and hsp65 gene.
Out of 244 M. intracellulare strains, 164were retrospectively undergoing to sequencing in ITS1 region.

Results: Out of 1037 NMT strains, 947 (91.3%) were identified by CM/AS kit for a total of 17 species with *M. intracellulare, M. xenopi* , *M. gordonae* , *M. avium*, *M. fortuitum* identified most frequently.
Out of 90 unidentified strains, 77 (85.5%) were identified by sequencing for a total of 21species with *M. arupense* identified most frequently. Among the 21 identified species, 11 were potentially recognizable by CM/AS (*M. abscessus*, *M. gordonae, M. avium*, *M. lentiflavum*, *M. xenopi*, *M. fortuitum*, *M. malmoense*, *M. peregrinum* , *M. chelonae, M. celatum,M. mucogenicum)*
The remaining 16 strains resulted *M.* spp by sequencing with 10 strains strongly related to *M. terrae* complex and 2 related to *M. fortuitum* complex.
Among the 164 *M. intracellulare* strains, only 27.5% were confirmed by sequencing. The other strains resulted *M yongonense* (23.8%), MAC (22.0%), *M. chimaera* (20.0%), *M. marseillense* (5.5%), *M. boucheduronense* (0.6%) and *M. fortuitum* (0.6%).

Conclusions: The GenoType Mycobacteria CM/AS kit allowed the identification of the most of NTM strains. This method can be considered excellent for the identification of a limited number of species circulating in our study area. The number of species detected by CM/AS and sequencing was similar (17 vs 21). Eleven out of 21 species identified by sequencing were potentially recognizable by CM/AS kit.Sixteen unidentified M. spp strains will be further investigated.
The ITS-1 sequencing of M. intracellulare strains showed in 72.4% the cross-reactivity of the CM9 probe with species unrecognized by kit. This has allowed the identification of strains recently

described as well as providing a very clear picture of the epidemiology of infections due to NTM in our clinical context.