Molecular methods and their role in the taxonomic reclassification of Nocardia spp. preserved in a culture collection

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Abstract:

The identification of the species belonging to the Nocardia genus, habitually is carried out according to laborious and time consuming conventional methods. The aim of this work was to use molecular methods in order to identify and reclassify taxonomically clinical isolations of Nocardia spp. preserved in a culture collection. Nineteen (19) Nocardia spp. identified by phenotypic conventional methods at the Culture Collection of the Mycology Department of the Instituto Nacional de Higiene Rafael Rangel were studied; these isolates were sent later to the Laboratory of Pathogenic Actinomycetes to corroborate its identification, also using phenotypic methods. Nocardia spp. isolates were preserved under mineral oil and by the Castellani method during a time interval of 2 to 50 years, before the accomplishment of the molecular studies. The genomic DNA was extracted using the achromopeptidase method, and a Nocardia-genus specific PCR was conducted, amplifying a 606 bp-determined region of the 16S rRNA, using the primers Noc1 and Noc2. The PCR products were sequenced and the obtained sequences were sent to the GenBank database. Of seven N. brasiliensis, one was identified as N. otitidiscaviarum, and another one as N. asteroides type IV by sequencing. Of five N. otitidiscaviarum, one was identified as N. farcinica, and another one as N. mexicana. Of the seven N. asteroides isolates identified by conventional phenotypic methods, one was identified as N. asteroides type IV, two were identified as N. nova, and the others corresponded to N. otitidiscaviarum, N. veterana, N. farcinica, and N. cerradoensis. The use of molecular methods contributed to new findings and reclassify taxonomically eleven clinical isolates, five of which corresponded to new causal agents of nocardiosis and actinomycetoma first time isolated in Venezuela, which directly influences in the epidemiology of the Nocardia spp. infections in our country. The culture collections play a crucial role in the ex- situ conservation, serving like repositories of microorganisms. For this reason, they must be in the capacity to provide authentic taxonomic identification of the microorganisms that they preserve. It is very important to preserve clinical isolates of Nocardia spp. in culture collections, so that they can be better studied at phenotypic and molecular level.

Key words: culture collections, molecular taxonomy, Nocardia spp.