TITLE: MOLECULAR CHARACTERIZATION OF NEW *Corynebacterium* SPECIES ISOLATED FROM UROCULTURES

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ABSTRACT:
Although the reports of infections in humans by *Corynebacterium* spp. have increased, identification at the species level in diagnostic laboratories remains a challenge and the usual negligence, even because they are microorganisms belonging to the microbiota of human skin and mucosa. Gene sequencing and MALDI-TOF mass spectrometry are proving to be increasingly valuable tools, as they allow the identification, quickly and reliably, of several bacterial species, even uncommon and rare ones. Thus, to perform a performance verification, in the present work, five coryneform isolates recovered from the urinary tract were identified by different methods: biochemical conventional tests, API® Coryne commercial system, MALDI-TOF mass spectrometry (Microflex®, Bruker Daltonics) and gene sequencing, 16S rRNA and *rpo*B (ABI PRISM 3100 DNA Sequencer, Applied Biosystems®). Conventional phenotypic tests and the API® Coryne system only allowed identification at the genus level. The MALDI-TOF system identified two isolates as *Corynebacterium aurimucosum* (score ≥ 2,000) and the others only at the genus level (score < 2,000). Regarding the analysis of sequence similarity of the 16S rRNA gene, the results were inconclusive, as the values above the proposed cutoff point of 98.7% were obtained for two or more species of (C. aurimucosum, *Corynebacterium singulare*, and *Corynebacterium minutissimum*). Finally, the analysis of the similarity of the *rpo*B gene, considering the proposed cut-off point of 95%, directed the isolates to the species *C. aurimucosum*. The CLUSTAL X2 and MEGA X programs were used for the alignments and construction of phylogenetic trees, respectively and, in this context, the phylogenetic analysis of the 16S rRNA and rpoB genes revealed that the isolates are grouped into distinct clades, phylogenetically related to *C. aurimucosum*, composing, probably, two new species of the genus. Additional taxonomic analyzes are needed for the formal description of these species. Therefore, the results show that the database of the MALDI-TOF system and the cutoff points for species differentiation by the *rpo*B gene sequence need to be continuously updated so as not to compromise the identification of isolates, especially given the increasing characterization of new taxa.

Keywords: *Corynebacterium* spp., genome sequencing, MALDI-TOF, molecular characterization, phylogenetic analysis

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