A search for cryptic *Aspergillus* species in South Brazil

Búsqueda de especies crípticas de Aspergillus *en el sur de Brasil*

Dear Editor,

Cryptic *Aspergillus* species have become more relevant in clinical practice due to their increased resistance to first-line antifungal agents.[1] Mycologists used to rely on morphological characteristics to identify fungi at the species level, but in the last decade molecular analyses of DNA have been used for achieving a correct identification.[8] Such methodologies are not yet widely used in developing countries such as Brazil. Therefore, several clinical isolates belonging to *Aspegillus* genus were identified by sequencing the β-tubulin gene in order to find cryptic *Aspergillus* species.

Aspergillus isolates were obtained from cultures of clinical samples of patients attending Santa Casa de Misericordia de Porto Alegre for any medical reason between June and December 2019. Fungal DNA was extracted from pure cultures using Maxwell® 16 Blood Nucleic Acid Purification Kit (Promega, Brazil), and β-tubulin gene was amplified using, as previously described,[2] the following primers, purchased from Invitrogen (ThermoFisher Scientific, Brazil): GGTAACCAAATCGGTGCTGCTTTC (forward) and ACCCTCAGTGTAGTGACCCTTGGC (reverse). The amplification products were analyzed in 1.5% agarose gels. Amplicons were purified using ExoSAP-IT (Applied Biosystems) and sent for DNA Sanger sequencing. Sequencing results were aligned by ClustalW in the MegaX software, and the contigs were used for BlastN with NCBI database in search of the most similar sequence using parameters of percentage of identity >99% and E-value of 0.0.

Throughout six months, twenty isolates of *Aspergillus* were included in the study, but some had to be excluded due to the molecular results: four had many unresolved bases that impaired the proper assemble of the contigs, and very short readings (2–15 nucleotides) were obtained from the other three. Of the 13 isolates identified, *Aspergillus fumigatus* represented 69.2% of the cases (GenBank accession numbers MW478809, MW478810, MW478811, MW478812, MW478813, MW478814, MW478815, MW478818, MW478819) and *Aspergillus niger* 30.7% (GenBank accession numbers MW464654, MW478816, MW478817, MW478820).

Molecular techniques have become the cornerstone for the accurate identification of moulds at the species level. Despite that, such technologies are frequently not afordable for the clinical mycology laboratory. Only two studies have evaluated the frequency of cryptic species in hospital settings in Brazil. One of the studies evaluated a large culture collection made of *Aspergillus* isolates obtained from 12 medical centers, and found that cryptic species represented 19% (25/133) of the strains, with 90% of this cryptic isolates showing susceptibility to the three triazoles tested.[4] A study evaluating the epidemiology of *Aspergillus* in patients with cystic fibrosis found that 96% (51/53) of the isolates recovered were *A. fumigatus sensu stricto*, but no cryptic species were identified.[6] Multinational studies show that the prevalence of *Aspergillus* is around 10–15%.[5] Studies describing cryptic species of *Aspergillus* spent a long time for collecting the isolates or recruiting multiple centres, which resulted in a large number of strains.[5,9] Some studies recommend the use of more targets to identify cryptic species of *Aspergillus*, such as ITS, considered a universal target for filamentous fungi, and calmodulin (CaM), which is a more specific marker, as β-tubulin, and the combination of at least two of these markers shows better performance in the identification of cryptic species.[3,7] This is the first study to evaluate *Aspergillus* cultures from South Brazil using fungal DNA sequencing. Cryptic *Aspergillus* species were not found, which is probably related to the small sample size.

In conclusion, the characterization of fungal genes, such as β-tubulin, allow the proper identification of the *Aspergillus* at the species level. Hospitals should check their own culture collection to determine the frequency of cryptic species in the *Aspergillus* genus, as well as within other medically important fungal species complexes, particularly if antifungal resistance is already described.

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