Screening of cryptic species among clinical Aspergillus isolates collected during one year period in a Portuguese reference laboratory

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ABSTRACT
Correct identification of Aspergillus species is important given that sibling species may show variable susceptibilities to multi antifungal drugs and also because sharper definition of species may facilitate epidemiological studies. Thus, we screened Aspergillus clinical isolates from Portuguese hospitals to determine which, if any, of the cryptic species of Aspergillus were involved in patient infections.

Over a one year period, Aspergillus isolates from Portuguese health institutions were collected. These isolates were identified on the basis of morphological and through the use of molecular tools. Genomic DNA was prepared from each isolate and the sequencing of the Internal Transcribed Spacers (ITS) regions, specifically the ITS1 and ITS2 non-coding regions flanking the 5.8S rDNA was used to determine the species complex, whereas β-tubulin and calmodulin sequencing was done to achieve the correct species identification.

Over the study period, 57 Aspergillus isolates from clinical samples were collected from 10 Portuguese health institutions. According to the morphological observations, 29 isolates were identified as Aspergillus fumigatus; 11 A. flavus, 8 A. niger, 3 A. nidulans, 2 A. terreus, 2 A. candidus and 2 Aspergillus sp. Among those isolates, six species-complexes were detected by ITS sequencing, and were distributed as follows: Fumigati (50.1%), Flavi (21.0%), Nigri (15.8%), Terrei (5.3%), Nidulantes (3.6%) and Versicorales (3.6%). β-tubulin and calmodulin sequencing resulted in ten (17.5%) cryptic species being identified among the 57 isolates. Seven of those isolates belonged to the Nigri complex (A. brasiliensis, A. awamori and A. tubigenes), two to the Versicorales complex (A. sidowii and A. fruticis), one to the Fumigati complex (A. lentulus) and one to the Nidulantes complex (Emmericella echinulata).

With rigorous application of molecular tools, cryptic species of Aspergillus are not uncommon in the clinic. The identification of cryptic species among the collected clinical isolates of Aspergillus alerts the clinician to isolates with reduced susceptibilities to antifungal drugs and emphasizes a correct identification to species level.

INTRODUCTION
Correct identification of Aspergillus species is important given that sibling/cryptic species may show different susceptibilities to antifungal and also because sharper definition of species may facilitate epidemiological studies.

OBJECTIVE
Clinical isolates of Aspergillus from Portuguese hospitals were screened to determine which, if any, of the cryptic species of Aspergillus were involved in patient infections.

METHODS
Over a one year period, isolates of Aspergillus were collected from 10 health institutions in Portugal.

All isolates were plated for growth as single colonies on malt extract agar (2%) with chloramphenicol (0.05 g/L). These isolates were identified on the basis of macro and microscopic morphology and through the use of molecular tools.

Genomic DNA was prepared from each isolate and the sequencing of the Internal Transcribed Spacers (ITS) regions was performed to achieve the basis of macro and microscopic morphology and through the use of molecular tools.

RESULTS & DISCUSSION

- A total of 57 Aspergillus isolates were collected.
- Among the collected isolates, six species-complexes were detected by ITS sequencing, and were distributed as follows: Fumigati (29 isolates; 50.9%), Flavi (12 isolates; 21.0%), Nigri (9 isolates; 15.8%), Terrei (3 isolates; 5.3%), Nidulantes (2 isolates; 3.6%) and Versicorales (2 isolates; 3.6%) (Table 1).
- Three misidentifications at the species-complex level (based on morphology) were resolved by ITS sequencing (isolates 12-59, 12-60 and INSA9) (Table 1).
- Isolates no. 12-47 and INSA7 were identified morphologically only to the genus level (Aspergillus sp.), but both were classified as belonging to the Versicorales complex by molecular methodologies.
- Eleven isolates (19.3%) belonging to seven Aspergillus cryptic species were identified (Table 1). Six of those isolates belonged to the Nigri complex (cryptic species A. brasiliensis, A. awamori and A. tubigenes), two to the Versicorales complex (cryptic species A. sidowii and A. fruticis), one to the Fumigati complex (cryptic species A. lentulus), and one to the Nidulantes complex (cryptic species Emmericella echinulata).

CONCLUSION
Cryptic species of Aspergillus have significant prevalence in the clinic. The identification of Aspergillus cryptic species among the collected clinical isolates emphasizes the importance of a correct identification to species level, since sibling species may have different antifungal susceptibilities.