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Identification of *Aspergillus* cryptic species in hospital environment

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Objectives Invasive aspergillosis is a fungal infection caused by *Aspergillus* spp. affecting mainly immunocompromised patients. The mortality rate can reach 85%. *Aspergillus* identification should be based on molecular methods as there are species morphologically similar but distinct at the molecular level (cryptic species), with variable antifungal susceptibility profiles. Recent studies have shown that cryptic *Aspergillus* species can cause approximately 10% of the cases of invasive aspergillosis. Since *Aspergillus* infections in immunocompromised patients are mainly nosocomial, knowledge of the fungal epidemiology found in hospital environments would have an important role in controlling the development of aspergillosis. Therefore, selected hospital wards, housing patients at higher risk to develop invasive fungal infections, were screened in order to understand the epidemiology and distribution of *Aspergillus*, especially regarding the presence of cryptic species.
Methods During a 1-year period, four seasonal samplings, i.e., air and hard surface, were performed. A total of 101 air samples and 99 surface samples were collected from the Hematology, Oncology, and Intensive Care Unit (ICU) wards of a Portuguese Central Hospital. *Aspergillus* isolates were plated for growth as single colonies on malt extract agar with chloramphenicol to check the colony purity. These isolates were identified on the basis of 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, 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.
Results 548 environmental fungal isolates were obtained. Of these, *Aspergillus* was the most frequently isolated genus (19.7%) and from the total of *Aspergillus* isolates, 75 were screened for cryptic species detection. The remaining *Aspergillus* isolates were not speciated either because viability was lost, contaminants were impossible to eliminate or amplification remained unsuccessful. Six misidentifications at the species-complex level (based on morphology) were resolved by ITS sequencing. This methodology allowed the identification of ten different sections within the *Aspergillus* genus: *Versicolores* ($N = 20$), *Nigri* ($N = 11$), *Flavi* ($N = 10$), *Circumdati* ($N = 10$), *Fumigati* ($N = 8$), *Usti* ($N = 4$), *Terrei* ($N = 4$), *Nidulantes* ($N = 4$), *Aspergilli* ($N = 3$) and *Cremei* ($N = 1$). From those, 25 different *Aspergillus* species were identified by β-tubulin and calmodulin sequencing, and a high percentage of cryptic species (i.e., not *sensu stricto*) was found (59%). Sections *Usti*, *Versicolores* and *Circumdati* harbored the highest proportion of cryptic species [100% (4/4), 95% (19/20) and 90% (9/10), respectively].
Conclusion The high number of cryptic species found raises concerns about the possible reduced susceptibility to antifungals of hospital environmental *Aspergillus* isolates. These data reinforce the importance of hospital air and surface monitoring, mainly in immunocompromised patients' wards. The knowledge of the *Aspergillus* epidemiology in hospital settings and the use of routine susceptibility testing will allow the monitoring of the rate of resistance in environmental strains and its potential impact on initial antifungal choices and therapeutic outcome.

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Epidemiology of *Candida pelliculosa*, *Candida utilis* and *Candida fabianii* in the Czech Republic

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Objectives Clinical yeast isolates belonging to *Candida pelliculosa*, *Candida utilis* and *Candida fabianii* are difficult to differentiate in a routine mycology laboratory using standard commercial biochemical kits. During the past decade, the use of invasive procedures and administration of antimicrobial agents and new technologies such as bone marrow transplants or chemotherapy have resulted in an increase in the incidence of non-*albicans* infections such as these three species. The aims of this study were (1) to determine the prevalence of *C. pelliculosa*, *C. utilis* and *C. fabianii* in clinical samples collected from 10 Czech hospitals using the biochemical kit ID 32C (bioMérieux) and MALDI-TOF mass spectrometry (Bruker Daltonics) and (2) to compare their minimum inhibitory concentrations (MICs) for 9 antifungals from various aspects.
Methods Two hundred and fifty-seven clinical yeast isolates were included in this study. Type strains of *C. pelliculosa* (CBS 605), *C. utilis* (CBS 841) and *C. fabianii* (CBS 5481) were added as controls. The whole group was first identified using ID 32C and then by the MALDI-TOF MS system. Identification of each strain was repeated in triplicate by both methods. In case of questionable identification, a sequencing analysis was performed. MICs of the systemic antifungals amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, micafungin, caspofungin and flucytosine were determined using the commercially available colorimetric broth dilution panels Sensititre YeastOne (TREK Diagnostic Systems). The results were compared with respect to patients' age, gender and site of infection and location of the hospital.
Results From a total number of 257 clinical isolates, 179 were biochemically identified as *C. pelliculosa*, 77 as *C. utilis*and 1 as *Williopsis saturnus*. The type strain of *C. fabianii* was determined as *C. pelliculosa*. Using MALDI-TOF MS confirmed with sequencing, 228 isolates were identified as *C. fabianii* (88.7%), 21 as *C. pelliculosa* (8.2%), 6 as *C. utilis* (2.3%) and 2 as *Ogataea polymorpha* (0.8%). The mean MICs ($\mu g\ ml^{-1}$) after 48 h were as follows: amphotericin B 0.77 (range, 0.12–2.0), anidulafungin 0.14 (0.015–2.0), micafungin 0.08 (0.008–1.0),caspofungin 1.17 (0.03–8.0), 5-flucytosine 7.61 (0.06–64.0),posaconazole 1.20 (0.03–8.0),voriconazole 0.21 (0.008–8.0), itraconazole 1.0 (0.03–16.0)and fluconazole 8.57 (0.5–256.0).The highest mean MICs were found in yeasts isolated from blood cultures and central venous catheters. No significant differences in MICs between genders were found.
Conclusion This study showed that, unlike routine biochemical identification, MALDI-TOF MS found *C. fabianii* to be most prevalent in clinical samples as compared with the other studied species. The absence of *C. fabianii* in databases of commonly used commercial biochemical kits for yeasts including ID 32C leads to misidentification of this species. In addition, some strains resistant to two or more antifungals were detected.
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