24 *ASPERGILLUS* NOSOCOMIAL INFECTIONS - DO CRYPTIC SPECIES FOUND IN HOSPITAL ENVIRONMENT MATTER?

R Sabino[1]*, C Viegas[2], C Veríssimo[1], M Francisco[1], C Martins[3], KV Clemons[4,5], DA Stevens[4,5]

[1] *National Institute of Health Dr. Ricardo Jorge – URSZ - Infectious Diseases Department, Lisbon, Portugal*
[2] *Scientific Area of Environmental Health, Lisbon School of Health Technology, Polytechnic Institute of Lisbon, Portugal*
[3] *North Lisbon Hospital Centre, EPE, Lisbon, Portugal*
[4] *California Institute for Medical Research, San Jose, CA, United States*
[5] *Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University, CA, United States*

Purpose:
Aspergillus is a major threat causing nosocomial infections in immunocompromised patients, especially those subjected to transplantation. Until recently, species identification relied on morphological features. Advances in molecular methods allowed species identification through sequencing of specific genes, allowing high discrimination amongst isolates, which enables the genetic differentiation to species level of morphologically identical isolates. These are the so-called cryptic or sibling species. Different *Aspergillus* species have different susceptibilities to antifungals and several cryptic species have been described as less susceptible to specific antifungals. Therefore, we addressed the possible influence of hospital environmental isolates in the overall situation of *Aspergillus* antifungal resistance.

Methods:
During one year, 101 air and 99 surface samples were collected from Hematology, Oncology and Intensive Care units of the Portuguese Central Hospital of Lisbon. *Aspergillus* isolates were identified morphologically and by molecular methods. Genomic DNA was prepared from each isolate and the sequencing of the Internal Transcribed Spacers (ITS) regions was used to determine the species complex. Sequencing of the β-tubulin and calmodulin genes was done to achieve the correct species identification. Determination of the antifungal susceptibility of selected isolates was performed by microdilution (CLSI M38-A2). The antifungal agents studied were deoxycholate amphotericin B, itraconazole, voriconazole, and posaconazole.

Results:
From the 200 samples collected, 75 isolates of *Aspergillus* were isolated and identified to section by ITS sequence; cryptic species were identified by β-tubulin and calmodulin sequencing. Ten different sections within the *Aspergillus* genus were identified: *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 these, 25 different *Aspergillus* species were identified by β-tubulin and calmodulin sequencing, and a high percentage of cryptic species (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]. From the 75 isolates, 22 were tested for their antifungal susceptibility. Of the 8 *Fumigati* isolates, there was 1 cryptic species (*Neosartorya hiratsukae).* The *Circumdati*, *Versicolores* and *Nigri* complexes contained isolates of cryptic species with reduced susceptibility to some of the antifungals used in clinical therapeutics. In the *Circumdati* complex, 3/8 isolates had MIC to amphotericin B >8μg/ml (*A. westerdjikae*) and 1/8 MIC >8μg/ml to itraconazole (*A. sclerotium*); 1/5 isolates from *Versicolores* complex had MIC to itraconazole >8 μg/ml (*A. sidowii*); all 4 isolates from *Nigri* (*A. tubigensis*, *phoenicis* and *niger sensu stricto*) complex had MIC to itraconazole= 4 μg/ml.

Conclusion:
Although aspergillosis caused by cryptic species remain less frequent than infections caused by "*sensu stricto*" isolates, in a recent study of *Aspergillus* clinical isolates collected in different Portuguese health institutions, we found that 19% of those were cryptic species. Since *Aspergillus* infections are mainly nosocomial, the knowledge of the molecular epidemiology and determination of the susceptibility profile of environmental isolates, which have a much higher frequency of cryptic isolates, may allow preventive or corrective measures to be taken. As a consequence, a decreased exposure to those organisms and a better prognosis is expected.