Surveillance of environmental fungi, with focus on *Aspergillus*, in a Portuguese Central Hospital

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Abstract

Because immunocompromised patients are more prone to acquire nosocomial infections caused by fungi isolated from the environment, e.g. *Aspergillus*, this study aimed to screen the hospital environment for the presence of fungi and to understand their epidemiology in the different hospital wards analyzed.
During one-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 and observe colonial morphology. The universal fungal primers ITS1 and ITS4 were used to amplify DNA from all *Aspergillus* isolates, amplimers were sequenced, and isolates identified to the species-complex level. Statistical analyses were done using SPSS v15.0 program for Windows.
***Aspergillus* was the most frequently recovered fungal genus (19.7%), followed by *Cladosporium* (18.6%), and *Penicillium* (17.3%). Thirty-five Aspergillus isolates were collected from the wards with hematological patients (bone marrow transplant and hemato-oncology wards), whereas 15 isolates were recovered from ICU. Among Aspergillus isolates from the hospital environment, those belonging to the species-complexes of Versicolores (n = 26; 32.5%), Nigri (n = 12; 15.0%), Flavi (n = 11; 13.7%), and Circumdati (n = 6; 7.5%) dominated. Hemato-Oncology was the ward with higher fungal counts, whereas the bone marrow transplant ward, which is protected by HEPA-filtration of the supply air, showed the lowest numbers in all sampling periods. A significant association (p = 0.001) was found between the season and the *Aspergillus* complexes isolated, with spring and summer having a larger number of different species-complexes detected in the hospital´s air and on the surfaces. Nevertheless, air counts showed that the autumn was the season with the highest proportion of *Aspergillus* (one third of the total number of fungi detected). This could be due in part to the presence of construction work near these wards.**
The knowledge of the epidemiology of environmental fungi in each hospital may allow the establishment of preventive or corrective measures to decrease nosocomial fungal infections.

Introduction

Invasive infections depend on the interplay between host susceptibility and environmental exposure to the organism. Because immunocompromised patients are more prone to acquire nosocomial infections caused by fungi isolated from the environment, e.g. *Aspergillus*, this surveillance study was performed in the hematology, oncology and ICU wards of a Portuguese Central Hospital.

Objectives

- ✓ To screen the hospital environment for the presence of fungi, especially *Aspergillus*
- ✓ To understand the epidemiology *Aspergillus* in the different hospital wards housing high risk patients.

Methods

- During four sampling periods (one year), 101 air and 99 surface samples were collected through impaction method and swabbing, respectively, from the Hematology, Oncology, and Intensive Care Unit (ICU) wards of a Portuguese Central Hospital.
- After plate incubation and colony isolation, microscopic mounts were made. Morphological Identification was done using macro- and microscopic characteristics.
- The fungal primers ITS1 and ITS4 were used to amplify DNA from all *Aspergillus* isolates, amplimers were sequenced, and isolates identified to the species-complex level.
- Statistical analyses were done using SPSS v15.0 program for Windows.

Results and Discussion

From all samples collected, ***Aspergillus* was the most frequently recovered fungal genus** (19.7%), followed by *Cladosporium* (18.6%), and *Penicillium* (17.3%) (Fig.1).

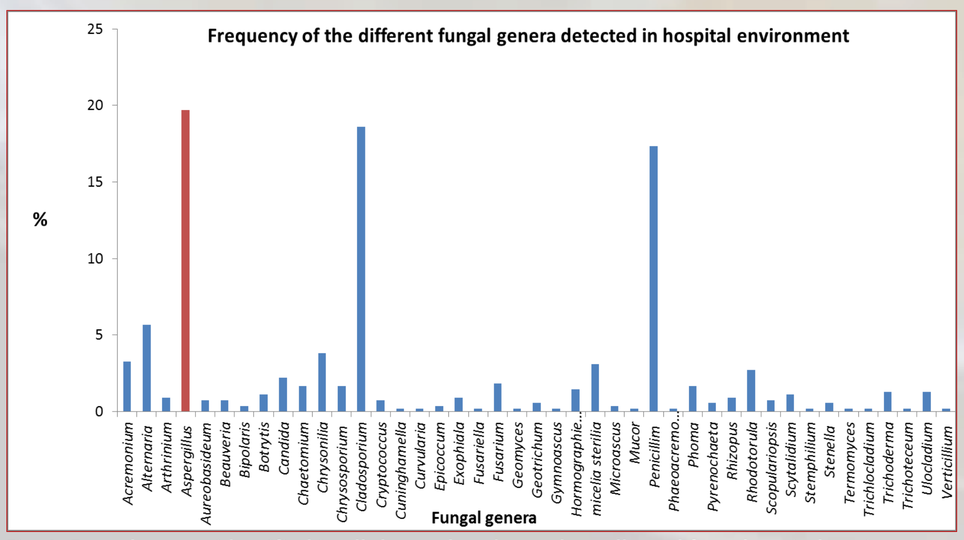

Fig. 1. Fungal genera identified in all the analyzed samples collected from hospital environment

- Twenty-three (28.8%) isolates of *Aspergillus* were collected during spring, 18 (22.5%) in summer, 23 (28.8%) in autumn and 16 (20.0%) in winter (Table I).
- A **significant association** (P = 0.001) was found between the **season** and the ***Aspergillus* complexes isolated**. Winter was the season with the lowest frequency of any of the species-complex under study, whereas spring and summer had a larger number of species-complexes detected.
- The ***Versicolores* complex was the only one recovered** in each of the **four sampling periods**, ranging from 2 to 28 cfu/m^3 of air (hematology ward) (Table I).

Table I. *Aspergillus* complexes' distribution along the different collection periods at hospital environment

Aspergillus complex	Spring (23 isolates)	Summer (18 isolates)	Autumn (23 isolates)	Winter (16 isolates)	Total
Aspergilli	1	0	0	4	**5**
Candidi	0	0	0	2	**2**
Circumdati	5	1	0	0	**6**
Flavi	5	1	5	0	**11**
Fumigati	0	3	3	1	**7**
Nidulantes	1	2	1	0	**4**
Nigri	4	6	0	1	**11**
Terrei	1	0	3	0	**4**
Usti	0	4	0	0	**4**
Versicolores	6	1	11	8	**26**

- Thirty-five isolates of *Aspergillus* were collected from wards with **hematological patients** (Fig. 2).
- In the **bone marrow transplant ward**, which is protected by HEPA-filtration of the supply air, only 5 isolates of *Aspergillus* were detected (complexes ***Fumigati*, *Terrei* and *Versicolores***), all from the **air** and most of them during the **autumn**.
- With respect to the **ICU** ward, 15 isolates were recovered; most were obtained from **surface** samples (Fig. 2). A predominance of these isolates belonged to ***Flavi*** complex.

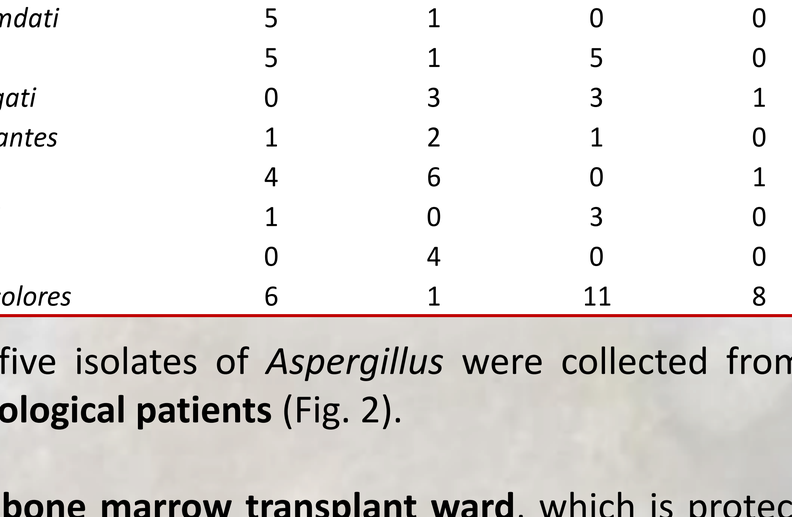

Fig. 2. Number of *Aspergillus* isolates collected from hospital environment in different hospital wards

Conclusion

The knowledge of the epidemiology of environmental fungi in each hospital may allow the establishment of preventive or corrective measures to decrease nosocomial fungal infections. The presence of *Aspergillus* must be assessed since immunocompromised patients are more prone to acquire nosocomial infections due to this fungus.

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