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# *Aspergillus* section *Fumigati* – Epidemiological trends. A perspective from a National Reference Laboratory

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## Abstract

*Aspergillus fumigatus* is the most frequent agent of aspergilosis and reports on infections caused by this species or its siblings are becoming more frequent. The overall objective of the study is to understand the epidemiology of the *Aspergillus* isolates (species and antifungal resistance) collected in the Portuguese National Reference Laboratory through our surveillance system on *Aspergillus*. During the period 2013-2017, 117 *Aspergillus* section *Fumigati* isolates were collected from 15 healthcare institutions of all country, and from different environmental sources .These isolates were identified on the basis of macro and microscopic morphology and through the use of molecular tools. Surveillance of azole resistance was performed firstly azole-resistant screening media, broth microdilution method or PCR for detection of mutations in the *Cyp51A* gene (when applicable). From the isolates collected during the study period, 94 were from clinical (human) sources, 2 from animals diagnosed with aspergillosis and 21 from environmental sources. In total, 111 *A. fumigatus* sensu stricto isolates were identified, followed by 3 *A. lentulus*, 2 *A. felis* and 1 *A. hiratsukae*. Regarding susceptibility, relevant and residual growths were obtained in azole resistance screening media . The positive results were then screened by microdilution method and also by detection of Cyp51A mutations resistances were not confirmed. The median MIC values were higher than what is described in other studies which may explain the growth in screening media and may suggest a local epidemiology.

## Introduction

*Aspergillus* is one of the major fungal threats to immunocompromised patients, causing significant morbidity and mortality, especially of solid organ transplant patients or recipients of allogeneic haematopoietic stem cell transplantation. *Aspergillus fumigatus* is the most frequent agent of aspergilosis and reports on infections caused by this species or its siblings are becoming more frequent, together with the increasing number of at risk patients. Nowadays, due to the rising concerns on emerging antifungal resistance, the epidemiological surveillance for clinical and environmental isolates is mandatory.

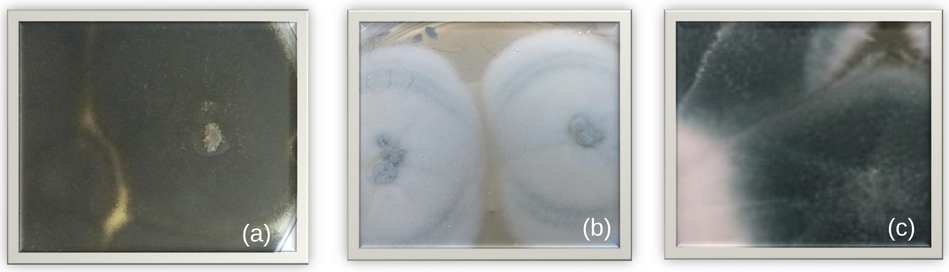


**Figure 1**: *Aspergillus* section *Fumigati*: (a) *Aspergillus fumigatus* sensu stricto; (b) *Aspergillus lentulus*; (c) *Aspergillus felis*

## Objectives

The overall objective of the project is to understand the epidemiology of the *Aspergillus* isolates (species and antifungal resistance) collected in the **Portuguese National Reference** Laboratory through our **surveillance system on *Aspergillus***.

## Methods

• During the period 2013-2017, 117 *Aspergillus* section *Fumigati* **isolates were collected** at the National Health Reference Dr. Ricardo Jorge, through the surveillance system on *Aspergillus*.

• All **isolates were identified** on the basis of macro and microscopic morphology and through ITS and calmodulin sequencing

• **Surveillance of azole resistance** was performed firstly using Sabouraud dextrose agar supplemented with itraconazole (ICZ), voriconazole (VCZ), and posaconazole (PCZ). When growth was observed, the minimal inhibitory concentration (MIC) was determined by broth microdilution method (CLSI M38A2). In case of doubt, a specific PCR for detection of mutations in the Cyp51A gene of *A. fumigatus* was performed using the AsperGenius® multiplex real-time PCR assay.

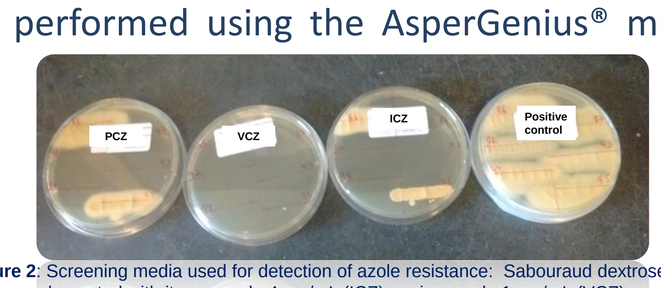


**Figure 2**: Screening media used for detection of azole resistance: Sabouraud dextrose agar supplemented with itraconazole 4 mg/mL (ICZ), voriconazole 1 mg/mL (VCZ), and posaconazole 0.5 mg/mL (PCZ).

## Results

• 94 isolates were from clinical (human) sources, 2 from animals diagnosed with aspergillosis and 21 from environmental sources (including hospital environment)

• Clinical isolates were obtained from 90 patients (53 males, 34 females, 3 not known), with ages ranging from 37 days to 88 years old. 98% of the isolates were from respiratory specimens. The underlying diseases reported are, among others, cystic fibrosis, COPD, HIV, asthma, and neoplasms.

• In total, 117 *A. fumigatus* (*sensu stricto* and cryptic species) were identified (Table 1). In 7 cases, the morphological identification did not matched with the correct species-section. Interestingly, the 5 clinical cryptic species (*A. lentulus* and *A. felis*) were from the same hospital.

**Table 1**. *Aspergillus* section *Fumigati* isolated during the study period

| Species molecular identification | Number of collected isolates | Source |
|---|---|---|
| *Aspergillus fumigatus* sensu stricto | 111 | Clinical and Environmental |
| *Aspergillus lentulus* | 3 | Clinical (respiratory specimens) |
| *Aspergillus felis* | 2 | Clinical (respiratory specimens) |
| *Aspergillus hiratsukae* | 1 | Hospital environment |

• Regarding susceptibility, relevant and residual growth were obtained in azole resistance screening media (Table 2). Positive results were then tested by microdilution method and by detection of Cyp51A mutations. The reference method did not confirm resistances and no Cyp51A mutations were found.

**Table 2**. Results of inoculation of the studied *Fumigati* isolates in Sabouraud dextrose agar supplemented with itraconazole 4 mg/mL (ICZ), voriconazole 1 mg/mL (VCZ), and posaconazole 0.5 mg/mL (PCZ).

| | ICZ (4 mg/mL) | | | VCZ (1 mg/mL) | | | PCZ (0.5 mg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Isolate growth in culture** | Relevant | Residual | Negative | Relevant | Residual | Negative | Relevant | Residual | Negative |
| *A. fumigatus* sensu stricto (N=104)* | 13 | 18 | 73 | 0 | 1 | 103 | 3 | 3 | 98 |
| *A. lentulus* (N=3) | 2 | 0 | 1 | 1 | 1 | 1 | 2 | 0 | 1 |
| *A. felis* (N=1) | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 |
| *A. hiratsukae* (N=1) | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |

*The remaining isolates lost their viability and did not grow in the positive control

## Conclusions

In our collection of ***Fumigati* isolates, 5% of them were cryptic species**. Although **no azole** resistance was found by microdilution or detection of Cyp51A mutations, the MIC values obtained suggest that the median values are higher than what is described in other studies (1.4 to ICZ, 0.4 to PCZ), which may explain the growth in screening media and may suggest a **local epidemiology different from other countries**.

•Alanio A, Cordonnier C, Bretagne S. Azole resistance in *Aspergillus fumigatus*—current epidemiology and future perspectives.*Curr Fungal Infect Rep* 2011; 5: 168–178
•Clinical Laboratory Standard Institute. "Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard.M38-A2. 2008; Clinical Laboratory Standard Institute, Wayne, PA, USA.
•Snelders E, van der Lee H, Kuijpers J et al. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 2008; 5: e219.
•Frédéric Lamoth F. *Aspergillus fumigatus*-Related Species in Clinical Practice. *Front Microbiol*; 2016; 7: 683.