#118: Frequency and molecular epidemiology of *Aspergillus* isolated from patients with suspicion of respiratory fungal infection

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ABSTRACT

We retrospectively analyzed a cohort of 146 patients with suspicion of respiratory fungal infection. The prevalence of *Aspergillus* was calculated, as well as the frequency of each section and species of these fungi isolated from respiratory samples. Resistance to azoles was determined for the *Aspergillus* of the *Fumigati* section.

Fifty-seven (39.0%) of the patients studied were positive for *Aspergillus*. Six sections and 13 different species of *Aspergillus* were detected, of which 9 were cryptic species. The *Nigri* section and the species *A. niger sensu stricto* were the most frequent. The *A. fumigatus sensu stricto* isolates tested did not show resistance to the azoles, but an *A. udagawae* strain revealed low susceptibility to voriconazole and an *A. pseudofelis* strain showed reduced susceptibility to voriconazole and itraconazole.

The genera *Aspergillus* was frequently detected in the respiratory samples tested and a great variety of species were identified. Surveillance of *Aspergillus* resistance should persist, although we only detected a decrease in the susceptibility of two cryptic species.

INTRODUCTION

In Portugal, it is estimated that there are approximately 12 600 cases of allergic bronchopulmonary aspergillosis. 776 cases of chronic pulmonary aspergillosis and 240 cases of invasive aspergillosis per year [1], but data about the epidemiology of these diseases is lacking.

Posaconazole, voriconazole and itraconazole are the standardized drugs used for prophylaxis and treatment of *aspergillosis* [2,3]. However, the use of these antifungals is being compromised by intrinsic resistance of some cryptic species of *Aspergillus,* as well as by the emergence of secondary resistance in *A. fumigatus sensu stricto.*

OBJECTIVE

We aimed to contribute to the surveillance of *Aspergillus* in Portugal by determining the prevalence and frequency of these fungi in respiratory samples from a cohort of patients with symptoms of respiratory fungal infection, as well as by determining the susceptibility to azoles of *Aspergillus* of the *Fumigati* section

METHODS

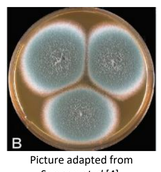
Collection of respiratory samples (Nov. 2011 – Dec. 2017) in 16 hospitals from different districts of Portugal

Sample type	Nr.
Bronchoalveolar lavages	101
Bronchial lavages	52
Bronchial secretions	27
Bronchial aspirate	1
Expectoration	6

→ **Detection of fungi by immunoenzimatic, molecular and cultural techniques**

Calculation of the prevalence of *Aspergillus* spp. In the cohort.

Positive culture for filamentous fungi

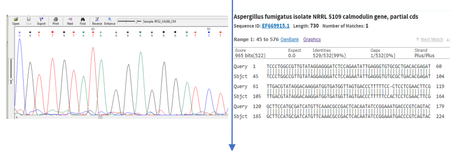


Picture adapted from Samson *et al.*[4].

→ **Morphological identification as *Aspergillus* spp.**

DNA extraction

→ **Species identification trough sequencing of the calmodulin/β-tubulin genes (n=58)**



→ Calculation of the frequency of each section and species of *Aspergillus* detected.

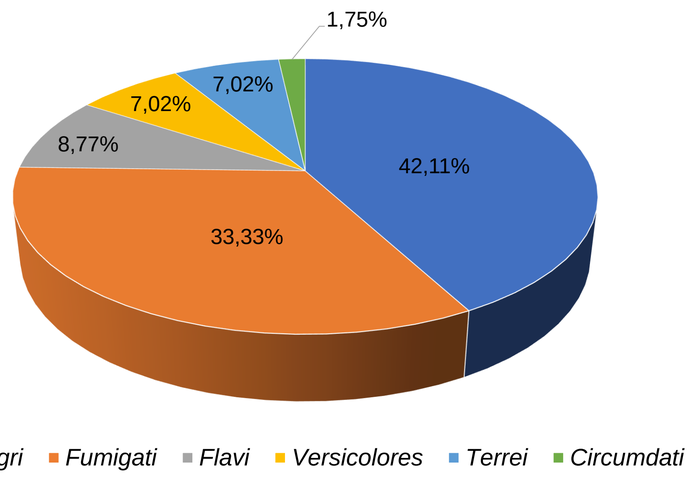
→ **Determination of the susceptibility to azoles of isolates (n=52) from the *Fumigati* section**

Inoculation in Sabouraud dextrose agar media supplemented with vori-, itra- and posaconazole.

RESULTS

A cohort of 146 patients with suspicion of respiratory fungal infection was studied and the presence of *Aspergillus* (n=187) in their respiratory samples was recorded (Figure 1A and B). **Thirty-seven of these patients (39.0%) were positive for *Aspergillus* spp.**

A) Frequency of each section of *Aspergillus* detected



B) Frequency of each species of *Aspergillus* detected

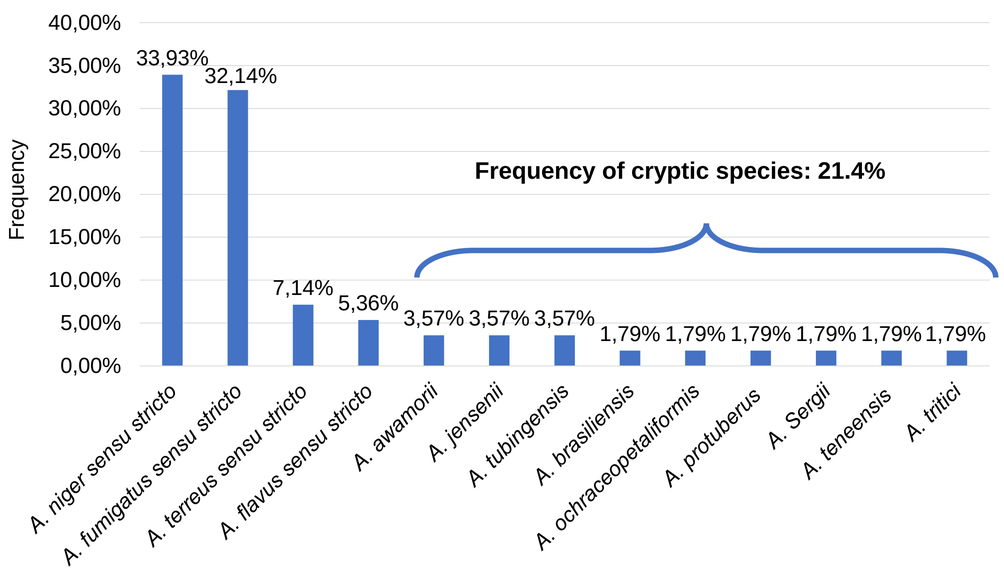


Figure 1 – Frequency of each section (A) and species (B) of *Aspergillus* identified.

Azole susceptibility of the 52 *Fumigati* isolates were tested by screening media.
Obtained results are exemplified in Figure 2 and summarized in Table 1.

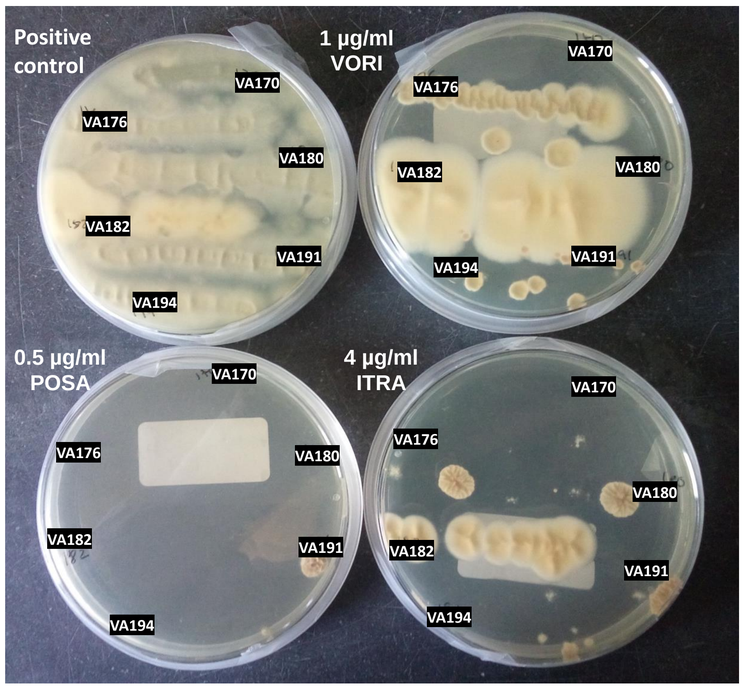


Figure 2 – Susceptibility screening of *Aspergillus* spp. The isolates VA170, VA176, VA180, VA191 and VA194 were identified as *A. fumigatus sensu stricto*. The isolate VA182 was identified as *A. pseudofelis*.
VORI – voriconazole; POSA – posaconazole; ITRA –itraconazole.

Table 1 – Number of isolates of each species with significative (+), residual (±) or negative (-) growth on the screening medium supplemented with voriconazole, itraconazole and posaconazole.

	Voriconazole (4 µg/ml)			Itraconazole (4 µg/ml)			Posaconazole (0.5 µg/ml)		
	Growth on the screening media			Growth on the screening media			Growth on the screening media		
Species	+	±	-	+	±	-	+	±	-
***A. fumigatus* sensu stricto (n=45)**	0	0	45	0	1	45	0	0	45
***A. lentulus* (n=4)**	0	0	4	0	0	4	0	0	4
***A. pseudofelis* (n=1)**	**1**	0	0	**1**	0	0	0	0	1
***A. udagawae* (n=2)**	**1**	0	1	0	0	2	0	0	3

FINAL REMARKS

- ✓ There is a high prevalence and a great variety of *Aspergillus* spp. colonizing/infecting the respiratory tract of patients with symptoms of respiratory fungal infection.
- ✓ Contrary to what has been previously reported worldwide - including in Portugal [5,6] -, the *Nigri* section and the species *A. niger sensu stricto* were the most frequent in the respiratory samples tested. This reveals the diversity and complexity of *Aspergillus* spp. distribution.
- ✓ Cryptic species were commonly isolated in the respiratory samples of this cohort and some of them - *A. pseudofelis* and *A. udagawae* - revealed a decrease in susceptibility to some of the azoles tested.
- ✓ *A. fumigatus sensu stricto* resistance to azoles was not described in this work, but surveillance should persist.

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