Aspergillus Section Fumigati in Firefighter Headquarters

Subjects: Public, Environmental & Occupational Health

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Aspergillus species are filamentous fungi commonly observed in different environmental compartments such as soil, water and air, with an emphasis on decaying vegetation, seeds and grains, where they prosper as saprophytes. Aspergillus species are also found in different indoor environments, and some species are considered opportunistic pathogens for humans. Aspergillus conidia can be abundant in outdoor and indoor environments and are easily dispersed in the air depending on the developed activities. Since the conidia are very small, they are easily inhaled and may colonize the upper and lower respiratory tract of exposed individuals. Aspergillus section Fumigati is one of the Aspergillus sections more frequently related to respiratory symptoms due to the small size of the conidia, thermotolerance, its nutritional versatility, and several other virulence factors . Additionally, the development of resistance to antifungal drugs, mainly in this Aspergillus section, is a phenomenon with growing prevalence in Europe, being associated with therapeutic failure and high mortality rates.

Keywords: sampling approach ; culture-based methods ; molecular tools ; azole resistance profile

1. Aspergillus Section Fumigati Distribution

Among all the firefighter headquarters (FFHs), and concerning *Aspergillus* genera, the highest value obtained by the Andersen six-stage air samples was observed on FFH5 (3.81%), and the same trend was obtained from the cleaning cloths and filters from the same FFH (0.51% and 7.33%). Concerning Millipore air samples, the FFH10 presented the highest counts (1.87%). *Fumigati* was the predominant section in Andersen six-stage FFH7 (88.37%), while for Millipore air samples, the highest counts were observed in FFH9 (100%).

In FFH4, the *Aspergillus* genera was predominant in EDCs (0.55%) and in settled dust (0.65%) samples, while in FFH2, the section *Fumigati* was the most frequent in EDCs (100%). *Aspergillus* sp. was identified in mops from FFH1 (0.44%), *Fumigati* being the predominant section (100%). Similar results were obtained in swabs samples from FFH3, *Aspergillus* being the most frequent genera (0.08%) and *Fumigati* being the prevalent section (100%).

Regarding samples collected from all FFHs, the genus *Aspergillus* was present in almost all matrices, with a prevalence of 1.52% in MEA (Millipore; six-stage Andersen; EDCs; cleaning cloths; mops; settled dust filters; swabs) and 2.20% in DG18 (Millipore; six-stage Andersen; EDCs; cleaning cloths; settled dust filters; swabs), being absent in mops, identification badges and settled dust samples in MEA and identification badges in DG18 (**Table 1**).

Table 1. *Aspergillus* sp. distribution in all matrices in MEA and DG18 from all FFHs.

Sample	MEA			DG18		
	Fungi	CFU. $m^{-3}/m^{-2}/g$	%	Fungi	CFU. m^3 $3/m^2/g$	%
Andersen	Other species *Aspergillus* sp.	405,281.8 486	99.9 0.1	Other species *Aspergillus* sp.	67,915.2 1040	98.5 1.5
Millipore	Other species *Aspergillus* sp.	147,399.8 158.2	99.9 0.1	Other species *Aspergillus* sp	50,624.64 393	99.2 0.8
EDC *	Other species *Aspergillus* sp.	391,345 743.1	99.8 0.2	Other species *Aspergillus* sp.	210,245.9 4640.126	97.8 2.2
Cleaning cloths	Other species *Aspergillus* sp.	29,500 500	98.3 1.7	Other species *Aspergillus* sp.	19,700 1500	92.9 7.1

Sample	MEA			DG18		
	Fungi	CFU. $m^{-3}/m^{-2}/g$	%	Fungi	CFU. m^3 $3/m^2/g$	%
Mops	Other species	2600	100	Other species	13,500	84.4
	Aspergillus sp.	-	-	*Aspergillus* sp.	2500	15.6
Identification badges	Other species	45,500	100	Other species	32,500	100
	Aspergillus sp.	-	-	*Aspergillus* sp.	-	-
Filters	Other species	3,679,700	96.6	Other species	2,699,000	98.1
	Aspergillus sp.	128,500	3.4	*Aspergillus* sp.	52,505	1.9
Settled dust	Other species	6496.5	100	Other species	2983.9	99.1
	Aspergillus sp.	-	-	*Aspergillus* sp.	27	0.9
Swabs	Other species	4,562,500	99.8	Other species	4,131,556	97.6
	Aspergillus sp.	10,000	0.2	*Aspergillus* sp.	100,000	2.4

* EDC (Electrostatic dust collector) results were presented in CFU.m^{-2}.day^{-1}; MEA—Malt Extract Agar; DG18—Dichloran–Glycerol Agar.

Of all the matrices, the highest counts of *Aspergillus* sp. were obtained on filters (3.37% MEA, 19.09% DG18) followed by cleaning cloths (1.67% MEA; 7.07% DG18). The matrices where the lowest prevalence was reported were air samples from Millipore (0.10% MEA, 0.77% DG18), six-stage Andersen (0.11% MEA) and settled dust (0.89% DG18) (**Figure 1**).

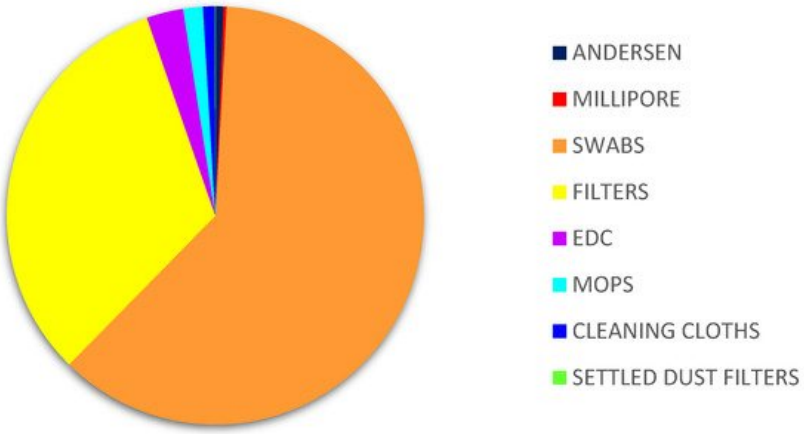

Figure 1. *Aspergillus* sp. distribution in samples on DG18. DG18: Dichloran–Glycerol Agar; EDC: Electrostatic dust collector.

Among the *Aspergillus* genus, the *Fumigati* section was predominant in Millipore and EDC samples in MEA (79.77% and 28.57%, respectively), and in swabs and settled dust filters in DG18 (44.76% and 30%, respectively). This section was also observed in six-stage Andersen samples (25.04% MEA; 16.99% DG18) (**Figure 2**). Among *Aspergillus* sp., section *Fumigati* was identified in the Andersen air sampler through all six stages. In FFH8, the section was the only one identified (100% on DG18) in stage 1 (7 µm). In FFH2, despite the lower frequency (4% MEA), the section was reported on the 2nd stage (4.7 µm). The section was the only one found on DG18 in stage 3 (3.1 µm) in FFH7. The same trend was obtained in FFH2 (100% MEA) in stage 4 (2.1 µm) and in FFH6 (100% MEA) in stage 5 (1.1 µm). In addition, FFH5, FFH6 and FFH10 had similar results (100% MEA) in stage 6 (0.65 µm). In stage 6, section *Fumigati* was the only found in the two culture media from FFH8 (100% MEA and DG18). Concerning the *Fumigati* section, positive samples (n = 23), the section was more frequently detected in samples performed by the Andersen sampling device (Table S3) and was more frequently detected in DG18 (33.01%) compared to MEA (0.33%) (Table S4).

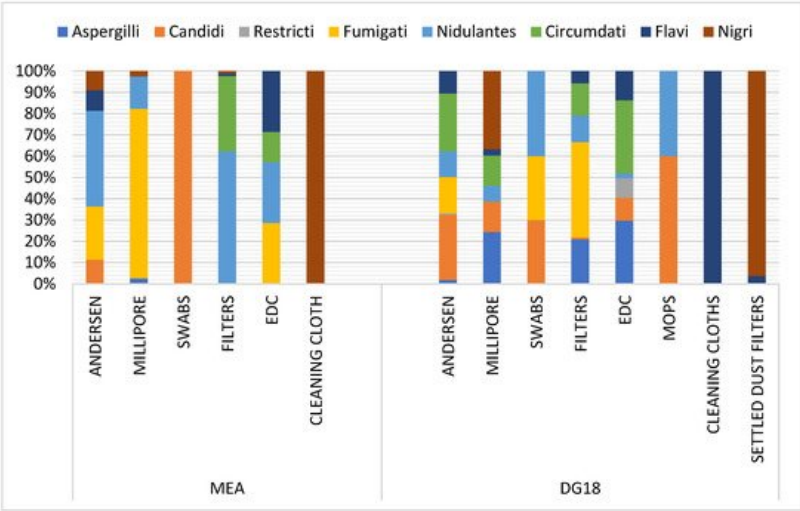

Figure 2. Distribution of *Aspergillus* sections per matrice in MEA (Malt Extract Agar) and DG18 (Dichloran–Glycerol Agar).

2. Screening of Azole Resistance

Passive matrices (82 EDC, 102 swabs, 89 filters, 11 settled dust, 67 uniform name tags, 25 cleaning cloths and 14 mops) were extracted as described and screened for antifungal resistance to three commonly used medical azoles. Growth of *Aspergillus* sp. was observed in Sabouraud (SDA: 1.11%) and in two azole-supplemented SDA media (ITR: 0.11%; VOR: 0.11%), with no growth observed in the POS media. The *Fumigati* section was the only among *Aspergillus* sp. observed in three culture media (SDA: 8.9%; ITR: 100%; VOR: 85.3%) (**Table 2**).

Table 2. Prevalence of *Aspergillus* section *Fumigati* among *Aspergillus* sp. in azole-supplemented SDA media.

Matrices	SDA		ITR		VOR		POS	
	CFU.m²	%	CFU.m²	%	CFU.m²	%	CFU.m²	%
Mops	2500	100%	-	-	-	-	-	-
EDC *	106	4.4%	1062	100%	3503	97.1%	-	-
Settled dust filters	1500	3.7%	1062	100%	-	-	-	-
Total	4106	8.9%	1562	100%	3503	85.3%	-	-

* EDC (Electrostatic dust collector) results were presented in $CFU.m^{-2}.day^{-1}$.; SDA (Sabouraud Dextrose Agar); ITR (Itraconazole); VOR (Voriconazole); POS (Posaconazole).

The relative frequency of azole-resistant *Aspergillus* section *Fumigati* isolates among all *Aspergillus* sp. isolates in the azole resistance screening was 17.8%. Isolates able to grow in 4 mg/L itraconazole and/or 2 mg/L voriconazole (all of them from *Aspergillus* genus) were identified in three different headquarters, from the following samples: 2 filters in FFH1; 1 EDC in FFH3; and 4 EDCs in FFH6.

Of all screened matrices, *Aspergillus* sp. was identified only in mops, EDCs, settled dust filters and settled dust. Concerning the *Aspergillus* sections, the *Fumigati* section was prevalent in the SDA of 100% in mops, 4.4% in EDCs and 3.7% in filters. *Fumigati* was the only section found in ITR (filters and EDCs: 100%) and the most predominant in VOR (EDCs: 97.1%) (**Table 2**).

The results of *Aspergillus* sp. relative distribution per sample type and culture media are depicted in **Figure 3**. The *Fumigati* section was predominant among *Aspergillus* sp. in mop samples in SDA media, abundant in EDCs and settled dust filter samples, including in ITR and VOR, and absent in settled dust.

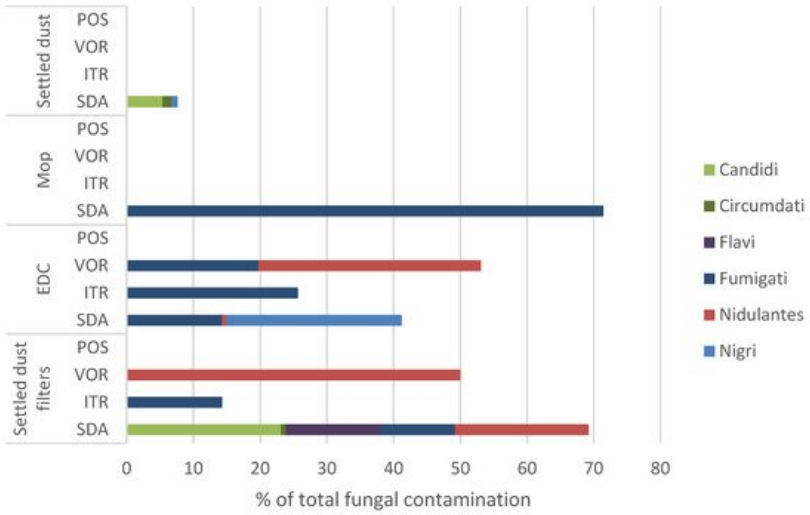

Figure 3. Relative distribution of *Aspergillus* sections per matrix type in SDA and azole-supplemented SDA media (ITR, VOR, POS) regarding total fungal contamination. SDA (Sabouraud Dextrose Agar); ITR (Itraconazole); VOR (Voriconazole); POS (Posaconazole); EDC (Electrostatic dust collector).

3. Discussion

The presence of fungi in an indoor environment is influenced by a wide range of variables, such as human occupancy and their activities, humidity levels, ventilation, environmental characteristics, water infiltrations, building and decoration materials and outdoor air [1][2]. Furthermore, there is strong scientific evidence corroborating the relationship between the building dampness, visible mould, and moisture damage with adverse respiratory health effects [3][4][5][6][7][8][9]. Moisture, nutrients and temperature are proved to be the most important variables that influence the growth and dissemination of fungi on building materials [10]. Thus, the results regarding *Aspergillus* sp. and *Fumigati* section contamination in the assessed FFHs were the expected considering the observed FFH conditions (in 8 from the 11 FFHs): leakages, visible mould growth and cracks on the walls/floor.

The identification of the *Aspergillus* section *Fumigati* through passive and active sampling has already been reported [3][11][12][13][14][15]. However, other studies developed in Portuguese occupational environments presented a lower prevalence of *Aspergillus* sp. and, more specifically, section *Fumigati* [16][17][18][19][12]. Indeed, in primary health care centres the *Fumigati* section presence was 33.3% on surface swabs and 1.3% in EDCs [16][17]. In one central hospital, *Aspergillus* sp. presented an overall prevalence of 17.25%, with the *Fumigati* section only being observed in the vacuum bag [18]; in Portuguese bakeries, the *Fumigati* section was found on air samples (3.2% on DG18) and in EDCs (8.3% on MEA and 50% on DG18) [19]; in three fitness centres, only four isolates of this section were found in one air sample [12].

In this study, a multiple approach protocol was performed comprising the two sampling methods for a better characterization of contamination in FFHs. *Aspergillus* counts were revealed to be higher in settled dust filters and cleaning cloths. Previous studies also identified passive sampling as suitable to determine *Aspergillus* section *Fumigati* by culture-based methods through sampling of filtering respiratory protective devices and other environmental matrices in settings such as waste-sorting plants, veterinary clinics, dairies, ambulances, and many other indoor and occupational environments [3][17][20][21][9]. In fact, passive sampling is able to characterize contamination levels over a wider period of time, compared to air sampling [22][13]. Thus, higher fungal counts and greater fungal diversity are expected by passive sampling.

The *Aspergillus* section *Fumigati* was predominant in swabs and settled dust filters in DG18, and in EDCs in MEA, suggesting that reliable matrices for *Aspergillus* section *Fumigati* exposure assessment were chosen [3]. The significant differences in fungal counts between passive and active sampling highlight the advantages associated with a multi-approach protocol that comprises active and passive sampling simultaneously, overcoming the limitations associated with each sampling method [3][23][16][17][24][18][19][25][26][20][27].

Regardless of the lower prevalence of *Aspergillus* sp. in air samples performed by Millipore and six-stage Andersen, the *Fumigati* section was predominant in Millipore air samples in MEA. Furthermore, the underestimation of microbial contamination collected by impaction devices (Andersen six-stage and Millipore sampling devices), due to cell damage during sampling process, has already been stated [21][28][29]. However, the six-stage Andersen sampler allows the hazardous range where the *Fumigati* section has lung penetrability to be identified [30][21][29]. Indeed, in four of the

assessed FFHs, section *Fumigati* at stage 6 (0.65 µm) of reaching alveoli was observed. This has the potential to cause respiratory diseases (inflammation activation) by activating macrophages, B cells and T cells [31]. The same concern was raised in a study held in Portuguese ambulances used in emergency clinical services [21].

Aspergillus section *Fumigati* was more frequent in DG18 compared to MEA counts. Despite the recommendation of using MEA for aerobiological studies in a Portuguese regulatory framework dedicated to the assessment of indoor air quality (IAQ) (25APA 2010), DG18 is an efficient option due to its restrictive character, inhibiting the development of fastidious fungal species [22]. The results of some fungal overgrowth on the MEA plates may have influenced the development of *Aspergillus* sp. and, more specifically, of the *Fumigati* section, due to chemical competition [32], highlighting the use of both culture media for a wider fungal characterization [3][23][16][17][24][18][19][25][26][20][27]. Sample dilution prior to inoculation (to avoid the excessive development of fast-growing fungi on media plates) was not performed due to the limitations of this option. Indeed, the removal of rare types of organisms leads to differences in species richness and diversity, decreasing competition among microorganisms, causing a probable overgrowth in some species that were not as prevalent in the original community [33].

The higher mean rank values obtained for total fungal contamination, *Aspergillus* sp. counts, and *Aspergillus* section *Fumigati* with SDA and ITR+VOR media, compared to MEA or DG18, suggest Sabouraud is a more suitable media for the recovery of fungi and *Aspergillus* sp. The use of Sabouraud as a standard medium to assess outdoor airborne fungi by air sampling was generally supported in a recent study on media comparison [34], and has also been supported in clinical applications [35]. However, Saboraud enhanced *Chrysonilia sitophila* in other performed assessments [16][21].

The ability to use protection against respiratory devices or filters as a sampling approach depends on the features of the assessed setting, activities developed and duration of use, e.g., mop sampling depends on cleaning procedures. The EDC device, on the other hand, allows for the recovery of fungal contamination in a consistent and standardized manner (regarding retention material and collection period), and it is a low-cost, low-maintenance sampling strategy that has been increasingly used in the assessment of occupational exposure to fungal burden [36] and in indoor air quality studies [22][9].

The fact that, among *Aspergillus* sections, only the *Fumigati* section was found in azole-supplemented media, confirms the presence of fungi potentially resistant to azoles in FFHs. If this azole-resistance phenotype is further confirmed by molecular analysis or antifungal susceptibility testing, it might represent a health risk for workers in this setting, especially in the FFHs where contamination by *Aspergillus* section *Fumigati* was higher. This health risk arises from the fact that azole-resistant fungi might cause invasive infections, especially in immunocompromised individuals, which are of difficult control due to the limited treatment options [37][3][38][36]. In addition to being the etiological agent of invasive aspergillosis, the *Fumigati* section is also responsible for more common respiratory symptoms such as asthma, allergic sinusitis, cough and bronchial hyperresponsiveness [39].

Culture-based methods allowed the *Aspergillus* section *Fumigati* to be identified in various matrices (settled dust filters, swabs, EDCs and air samples from Millipore and Andersen), confirming the results of molecular detection in EDCs and filters. The use of qPCR further enabled the *Fumigati* section to be detected in additional matrices (identification badges, mops and cleaning cloths) where it was undetectable by culture. This may be associated with the absence of fungal viability due to an impediment to grow in culture (e.g., due to competition for nutrients), while the molecular tools enable even non-viable microorganisms to be identified [40]. Failure to detect the *Aspergillus* section *Fumigati* by qPCR in swabs and air samples (in contrast to the results obtained by culture) may be associated with ineffective DNA extraction in sample processing, or the presence of inhibitors (such as particles from air samples), misleading the results [3][41][42]. Without diminishing the advantages of molecular analysis, classical culture-based methods are still necessary to assess the viability of pathogenic microorganisms related to their infectivity potential. Indeed, a microorganism's viability is associated to the potential of inflammatory and cytotoxic responses and, consequently, the infection potential. Therefore, molecular tools must be used in parallel with classic methods [3][43].

Correlation was found in this study between total fungal counts, *Aspergillus* sp. counts and *Fumigati* section counts not following the trend previously found in health care environments [16]. This means that the measures used to avoid fungal contamination in this setting are also effective concerning *Aspergillus* contamination. Nevertheless, *Aspergillus* genera assessment should always be performed, as specific *Aspergillus* sections (*Flavi, Fumigati, Circumdati* and *Nidulantes*) are indicators of harmful fungal contamination when found on air samples and require intervention, as referred to by the American Industrial Hygiene Association and Portuguese regulatory framework concerning IAQ [1][44].

Thus, considering the lack of scientific information in this specific environment, further studies are needed to characterize the overall exposure to fungal contamination and other microbiological agents, as well as regarding the most suitable

corrective and preventive measures used to avoid exposure. Additionally, further research on azole-resistance profile must be conducted to better estimate the risk of exposure to resistant *Aspergillus* section *Fumigati* in this setting, namely, screening azole resistance at selective conditions for *Aspergillus* section *Fumigati*, molecular analysis of resistance mutations, and antifungal susceptibility testing.

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