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Aspergillus prevalence in air conditioning filters from vehicles: taxis for patient transportation, forklifts, and personal vehicles

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Abstract

The frequency and importance of *Aspergillus* infections is increasing worldwide. This study aimed to assess the occupational exposure of forklifts and taxi drivers to *Aspergillus* spp. Nineteen filters from air conditioning system of taxis, 17 from forklifts and 37 from personal vehicles were assessed. Filters extract were streaked onto MEA, DG18 and in azole-supplemented media. Real-time quantitative PCR amplification of selected *Aspergillus* species-complex was also performed. Forklifts filter samples presented higher median values. *Aspergillus* section *Nigri* was the most observed in forklifts filters in MEA (28.2%) and in azole-supplemented media. DNA from *Aspergillus* sections *Fumigati* and *Versicolores* was successfully amplified by qPCR. This study enlightens the added value of using filters from the air conditioning system to assess *Aspergillus* spp. occupational exposure. *Aspergillus* azole resistance screening should be included in future occupational exposure assessments.

Key words: Aspergillus spp.; Air conditioning filter; Forklifts; Taxis; Occupational exposure assessment; *Aspergillus* azole resistance screening

Introduction

Aspergillus genus is widespread in the environment and human exposure to *Aspergillus* species is common. This genus is found in soil, dust, and decomposing organic matter and the conidia are often found in outdoor air.[1-4] Over the past 20 years, fungal infections of the lung, especially bronchopulmonary aspergillosis, have become increasingly common.[5-6] However, only a few well-known species are considered as important opportunistic pathogens in humans.[2,3] Thus, although there are more than 200 known species among the genus, only a small number is associated with infections in humans.[7] Polyphasic taxonomy has had a major impact on species definition among *Aspergillus* genus. The designations actually refer to sections (or complexes) of closely related species (also referred to as cryptic species) that cannot be clearly distinguished morphologically.[8] The genus has been subdivided into 22 distinct sections: *Aspergilli*, *Fumigati*, *Circumdati*, *Terrei*, *Nidulantes*, *Ornati*, *Warcupi*, *Candidi*, *Restricti*, *Usti*, *Flavipedes*, and *Versicolores* comprising clinically relevant species,[8,9] with the most relevant sections being *Fumigati*, *Flavipedes*, *Nigri* and *Terrei*.[10-12] The infection mechanism during aspergillosis has been addressed in numerous studies, with airborne infection by inhalation of *Aspergillus* conidia being a common assumption. However, the role of both *Aspergillus* load and duration of exposure to *Aspergillus* conidia in infection remain unclear.[13] In addition, the incidence of azole resistance in *Aspergillus* species has increased over the past years, essentially for *Aspergillus* section *Fumigati*.[14] Fungi from this section are the major cause of life threatening invasive aspergillosis (IA).[15]

High fungal contamination and the presence of potentially toxigenic fungal species in the waste industry have already been reported, with *Aspergillus* among the most prevalent fungus in this occupational environment.[16-21] A recently published pilot study focusing on eleven filters from the air conditioning system of forklifts cabinets in the Portuguese waste industry corroborates the same trend regarding *Aspergillus* prevalence.[22] On the contrary, little is known about the fungal burden inside taxi cabinets. Taxi cabinets are confined and often shared spaces and, because of that, taxi drivers and occupants face an increased risk of exposure to the bioburden in addition to other risk factors.[23-25] Of note, higher time of exposure for taxi drivers should be considered regarding a normal 8 hours' work shift, 5 days per week, than for other vehicle occupants.[26] Although studies developed either on bus or in personal vehicles rely mainly in fungal quantification,[27-29] they also cover fungal identification and report *Aspergillus* as one of the most prevalent genera present in this setting.[30-31]

Similar to indoor air conditioning systems in buildings, air filters in vehicles are intended to retain airborne bioburden, besides other organic dust components. Moreover, as other passive methods applied to assess occupational exposure to bioburden, they can collect contamination from a larger period of time (weeks to several months)[17-22] thus expressing a wider spectrum of the mycobiota.

This study aimed to assess the occupational exposure of forklifts drivers and taxi drivers to *Aspergillus*, through the compared evaluation of the bioburden in filters from the air conditioning systems from forklifts and taxis with filters from personal vehicles. Fungal assessment was performed applying culture-based and molecular methods, and the prevalence of *Aspergillus* species was determined.

Materials and methods

Filters from taxis and forklifts

Nineteen filters from air conditioning system of taxis, operating in patients transportation in three Portuguese cities (Lisbon, Loures and Setúbal), and seventeen filters from forklifts filtration system, operating in one waste sorting industry located in the Lisbon region, were analyzed. The taxi filters (made by carbon) were used during at least 15,000 kilometers, in agreement with the preventive maintenance program in the taxi company. The criteria for filter replacement in the air conditioning system was either dependent on the frequency established by the car brand to avoid filter blocking, as established in the preventive maintenance program, or dependent on visual observation by the maintenance service to accomplish preventive maintenance program.

The forklifts filters were used between 794 and 22,240 working hours and the ventilation provided for each vehicle cabinet was one cabinet volume/minute. In this case, the replacement of filters occurred earlier, since it was not possible to use the filter during the full time of the manufacturer recommendation due to the high visible fungal growth and filter blocking. These filters were composed by activated charcoal and belonged to category 2 (pores typically from 3.0 µm or greater dimension) according to protection requirements (EN 15695) ensuring protection against dust inside the cabinet. Forklifts were operating in an enclosed pavilion with several workplaces and operating the waste pile prior manual sorting by workers. The waste sorting plant (WSP) functioned 5 days/week in a daily regimen of two 8 hours shifts. None of the pavilions where forklifts functioned were provided with air conditioning, thus, being without temperature and humidity control.

Control filters

Thirty seven control filters from personal vehicles were assessed. The control filters, from personal vehicles, shared the same technical characteristics and replacement criteria than the taxi filters and probably complied only with the preventive maintenance program specific from each car brand.

Temperatures in Lisbon city range between 10 ºC (winter season) and 35 ºC (summer season). The forklifts filters were collected in summer, between July and August. Taxis and control filters were collected in winter, between January and March. After being removed, the filters were kept refrigerated at 4 ºC until extraction for analysis.

Culture based-methods

Two pieces of an area of each 2 cm^2 (1.4x1.4 cm) were cut from every filter. One piece of filter was extracted with 10 mL NaCl 0.9% aqueous solution with 0.1% Tween™ 80 in sterile 15 mL

falcon tubes for 30 minutes at 250 rpm on an orbital shaker (Edmund Bühler SM-30). Serial decimal dilutions of the extract were made with distilled water, and 150 µl were streaked onto 2% malt extract agar (MEA) with chloramphenicol (0.05 g/L) media, dichloran glycerol (DG18) agar based media, and Saboraud agar media supplemented with either 4 mg/L itraconazole, 1 mg/L voriconazole, or 0.5 mg/L posaconazole (adapted from the EUCAST guidelines[32]). Samples were incubated at 27 °C for 5–7 days, in order to allow the growth of all fungal species present in the samples.

After incubation at 27 ºC for 5 to 7 days, fungal densities (colony-forming units, CFU/m^2 of filter) were calculated, and fungal species were identified microscopically using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures. Morphological identification was achieved through macro and microscopic characteristics, as noted by De Hoog et al. (2000)[33] with recognition of morphologic characteristics from *Aspergillus* genus.

The other filter piece followed the same extraction procedure and 10 milliliters of the washed supernatant were frozen at -20 ºC for later DNA extraction.

Molecular biology

The frozen samples were thawed at room temperature then centrifuged at 3,500 × g for 30 minutes, the supernatant was removed and DNA was then extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research) according to the recommendations of the manufacturer. Molecular identification of the different species/strains was achieved by Real Time PCR (RT-PCR) using the Rotor-Gene 6000 qPCR Detection System (Corbett). Reactions included 1× iQ Supermix (Bio-Rad), 0.5 µM of each primer (Table 1), and 0.375 µM of TaqMan probe in a total volume of 20 µl. Amplification followed a three-step PCR: 40 cycles with denaturation at 95 ºC for 30 seconds, annealing at 52 ºC for 30 seconds, and extension at 72 ºC for 30 seconds. A non-template control was used in every PCR reaction. As positive controls of amplification, DNA samples were obtained from reference strains from the Mycology Laboratory from the National Institute of Health Doctor Ricardo Jorge (INSA).

Statistical analysis

Data analysis was performed using statistical software SPSS version 24.0. The results were considered significant at the 5%significance level. The Shapiro-Wilk test was used to test the normality of the data. Samples were characterized by using frequency analysis for the qualitative data and the calculation of the minimum, maximum, median and interquartil range for the quantitative data, since the normality assumption was not verified ($p < 0.05$). To compare fungal and *Aspergillus* spp. load (both for MEA and DG18) between the three filters samples (forklifts, taxis and controls) Krukal-Walllis test was used, since the assumption of normality did not occur.

Results

Aspergillus spp. contamination

Forklifts filter samples presented higher median values for total fungal load and *Aspergillus* spp. load in both media (Table 2), evidencing an increased fungal contamination in this group.

The prevalence of total fungal load in MEA was higher in forklifts filters (94.1%), followed by controls (54.1%) and taxis (22.2%). This pattern was also found in DG18, with total fungal load higher in forklifts filters (94.1%), followed by controls (45.9%) and taxis (38.9%) (Table 3).

Forklifts filters were the ones with higher contribution from *Aspergillus* spp. among the total fungal burden (59.5% MEA; 47.5% DG18), followed by controls filters (0.6% MEA; 0.5% DG18). Regarding taxis filters, *Aspergillus* spp. isolates were only identified in DG18 (18.8% DG18) (Figure 1).

Aspergillus section *Nigri* was the most observed in forklifts filters in MEA (28.2%), followed by *Fumigati* (26.6%) and *Circumdati* (25.8%). *Aspergillus* species belonging to *Aspergilli, Versicolores*, *Candidi* and *Flavi* sections were also identified (Figure 2). A different distribution was found on DG18 where *Circumdati* presented higher prevalence (51.7%), followed by *Nigri* (22.8%) and *Aspergilli* (19%). *Candidi* (6.2%) and *Flavi* (0.3%) were also observed in lower counts (Figure 2).

Regarding controls filters two *Aspergillus* sections were found in each media, namely sections *Nigri* and *Candidi* (50%) in MEA, and sections *Versicolores* (92.3%) and *Candidi* (7.7%) in DG18. In taxis filters only *Aspergillus* section *Candidi* was found in DG18.

Aspergillus *screening in azole-supplemented media*

Regarding forklifts filters, *Aspergillus* section *Nigri* was the most prevalent fungal species grown in azole-supplemented media (51%, 5,432 out of 10,683 isolates), as follows: 76% in 4 mg/L itraconazole, 43% in 1 mg/L voriconazole, and 0.33% in 0.05 mg/L posaconazole. The remaining *Aspergillus* species from forklifts filters observed in 4 mg/L itraconazole-supplemented media were *Aspergillus* section *Circumdati* (0.38%, 17 out of 4,497 isolates), *A*. section *Candidi* (0.31%, 14 out of 4,497 isolates), and *A*. section *Aspergilli* (0.02%, 1 out of 4,497 isolates). *Aspergillus* sections *Fumigati* and *Flavi* were only detected in non-supplemented media.

In taxis filters, the prevalence of fungal species in azole-supplemented media in was much lower, with only *Aspergillus* section *Candidi* identified in 4 mg/L itraconazole (50%, 3 out of 6 isolates). *Aspergillus* section *Nigri* was not detected in taxis filters in azole-supplemented media.

In filters from personal vehicles, no *Aspergillus* species were detected in azole-supplemented media (Table 4).

Aspergillus strains detection

DNA from toxigenic strains of the *Aspergillus* section *Flavi* and *Aspergillus* section *Circumdati* was not amplified by qPCR in none of the analyzed filters. However, DNA from *Aspergillus* section *Fumigati* was successfully amplified by qPCR in 7 forklifts filters and in 3 taxis filters. In three of the 7 forklifts filters it was also possible to detect DNA from *Aspergillus* section *Versicolores*, although at very low levels (high CT values). Of note, samples with lower cycle threshold (CT) values very likely exhibit higher levels of the *Aspergillus* sections targeted. Among the 10 filters where the target *Aspergillus* strains were detected, only in 1 forklift filter where the *Aspergillus* section *Fumigati* was also identified by culture based-methods.

Comparison analyses

Statistically significant differences were detected for the fungal load on MEA ($\chi^2_{K-W}(2) = 27.059, p = 0.000$), fungal load on DG18 ($\chi^2_{K-W}(2) = 30.331, p = 0.000$), *Aspergillus* spp. load on MEA ($\chi^2_{K-W}(2) = 57.960, p = 0.000$) and *Aspergillus* spp. load on DG18 ($\chi^2_{K-W}(2) = 25.778, p = 0.000$) between the samples groups. Multiple comparisons analysis allowed to detect differences between the forklifts filters group with the taxis group and with the controls group. It was also observed that the group with the highest load, either on total fungal load or on *Aspergillus* spp. load, was the forklifts filters (with the highest mean rank) and the group with lower fungal loads (MEA and DG18) and *Aspergillus* spp. load (MEA) was the taxis group (with lower mean ranks). As for the *Aspergillus* spp. load in DG18, the group with the lowest values was the controls, although very close to taxi group (Table 5).

Discussion

Aspergillus genus causes an extensive variety of infections comprising cutaneous manifestations, otomycosis, and invasive infections such as pulmonary aspergillosis and endocarditis.[37] The available information concerning *Aspergillus* infections is mainly related with the *Fumigati* section, the most frequent species complex.[38] The frequency and importance of *Aspergillus* infections is increasing in all industrialized countries.[39] Besides direct fungal load and contamination, exposure assessments should also consider a wider spectrum of the exposure burden, since almost all species complex among *Aspergillus* genus have toxigenic potential.[40] As such, and following the same trend of a previously suggested protocol, in order to ensure a proper occupational exposure assessment to *Aspergillus* spp., [21] filters from the air conditioning system were used as a passive method to assess the exposure to *Aspergillus* burden, through culture-based and molecular methods applied in parallel.

Fungal load and *Aspergillus* dominance on forklifts filters operating in waste industry reflect the same trend found in previously assessed worksites using filters[17] emphasizing the application of this passive method as an approach to be followed. Surprisingly, personal vehicles filters (controls) presented higher *Aspergillus* prevalence (besides other fungal species) than taxis filters (except for *Aspergilus* spp. load on DG18 where controls group exhibited less isolates), probably due to a higher frequency of filters substitution by the taxis company related to preventive maintenance program. A wider diversity of *Aspergillus* sections was found on forklifts filters and this occurrence can be due the availability of nutrients in

waste pile that is being operated by forklifts drivers.[22] Other variable that could had boost fungal growth was the fact that filters collection was made in summer, since optimum temperatures for fungal growth are around 25-30 ºC.[41]

Aspergillus section *Fumigati* was detected mainly in forklifts filters (7), which were heavily contaminated as corroborated by culture-based methods. It was also possible to detect this section in 3 taxis. *Aspergillus* section *Versicolores* was present, although at low levels (very high CTs) in 3 forklifts filters. Importantly, none of the *Aspergillus* sections were detected in control samples, indicating that their presence is specifically detected in highly contaminated filters. In addition, only in one filter from forklifts, from the 10 where detection of the target species was achieved, was possible to identify by culture based-methods the *Aspergillus* section *Fumigati*. This finding is in agreement with the protocol suggested for *Aspergillus* sp. burden assessment that recommends the use, in parallel, of both culture-based and molecular methods.[21]

The results also claim attention for the possible co-presence to mycotoxins and, consequently, the potential exposure to a mixture of mycotoxins, since all the *Aspergillus* sections detected have toxigenic potential.[42] Of note, although in lower counts than other *Aspergillus* sections, *Flavi* species complex was also observed in forklifts filters, therefore, reinforcing the workers biomonitoring results of occupational exposure to aflatoxin B1 (AFB1) already reported in the same waste management industry.[17] Viegas et al. (2015)[17] describe the presence of AFB1 in blood samples from all the enrolled workers (n=41) from the studied waste management industry. These results are of particular relevance since the control group (n=30) showed null results. Besides AFB1 others mycotoxins can also be present, namely, gliotoxin, ochratoxin A, sterygomisticin among others[42] since their main producers were also identified.

Regarding the prevalence of *Aspergillus* species in azole-supplemented media, an increased prevalence and wider species distribution of *Aspergillus* spp. were observed in forklifts filters. The waste industry had already been reported as having increased prevalence of *Aspergillus* genera.[21,22] The potential presence of azole fungicides, namely DMI (14-alpha demethylase inhibitors), in organic waste due to their amply use in farming in Europe and Asia for crop safety against phytopathogenic moulds[43-45] can exert some selection pressure in fungal population, potentially leading to the development of azole-resistance. In addition, since some of these DMIs (propiconazole, tebuconazole, difenoconazole, epoxiconazole and bromuconazole) reveal molecular characteristics very similar to the azole antifungals used for clinical purposes, cross-resistance to azole drugs can also develop.[46] Thus, it is currently being discussed worldwide whether fungal exposure to azole compounds coming from different environmental compartments can originate cross-resistance to medical triazoles.[45] A recent study reported that the acquisition of a mutation in the azole target by environmental strains provokes cross-resistance between the azole antifungals from the environment and the clinic.[47] In this study, *Aspergillus* section *Nigri* was the most observed among *Aspergillus* species recovered from filters grown in azole-supplemented media, mainly in 4 mg/L itraconazole. *Nigri* species have been reported in previous studies performed in clinical isolates as resistant to itraconazole.[48] Although increased itraconazole MIC´s have been described in

Nigri isolates, susceptibility data are relatively uncommon.[49,50] Of note, species belonging to the *Nigri* section are frequently reported to be the third most commonly occurring *Aspergillus* species related with invasive disease and aspergillomas.[51,52] The load of this species complex in forklifts filters, combined with his toxigenic potential and probable azole-resistance, represents a critical additional occupational hazard that should be tackled in future studies, in spite of being most of the times neglected.[53] Approaches limiting the emergence of azole-resistant strains, such as using fungicide products which cause less resistance, as well as manufacturing and marketing new and different azole products should be implemented to overcome this emergent occupational and public health menace.[54]

Conclusions

This study enlightens the added value of using filters from the air conditioning system from forklifts and taxis to assess the occupational exposure to *Aspergillus* spp. burden. It was possible to observe the same trend of fungal burden than the one obtained by using active methods for air sampling, and corroborates the biomonitoring data regarding mycotoxins occupational exposure in the waste industry. Applying culture-based and molecular methods proves to be an approach to be followed, also in filters from the air conditioning system, as a passive method applied for sampling to ensure a more real scenario concerning occupational exposure to mycobiota in general and to *Aspergillus* spp. in particular. The screening of *Aspergillus* azole resistance should be included in future occupational exposure assessments to fungal burden to unveil this occupational and public health threat.

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Conflict of Interest

None.

We have full control of all primary data and permission is given to the journal to review the data if requested.

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Table 1 - Sequence of primers and TaqMan probes used for Real Time PCR

Aspergillus **sections targeted**	**Sequences**	**Reference**
***Flavi* (Toxigenic Strains)**		
Forward Primer	5'-GTCCAAGCAACAGGCCAAGT-3'	
Reverse Primer	5'-TCGTGCATGTTGGTGATGGT-3'	(Mayer et al., 2003)[34]
Probe	5'-TGTCTTGATCGGCGCCCG-3'	
Fumigati		
Forward Primer	5'-CGCGTCCGGTCCTCG-3'	
Reverse Primer	5'-TTAGAAAAATAAAGTTGGGTGTCGG -3'	(Cruz-Perez et al., 2001)[35]
Probe	5'-TGTCACCTGCTCTGTAGGCCCG -3'	
Circumdati		
Forward Primer	5'-CGGGTCTAATGCAGCTCCAA-3'	
Reverse Primer	5'-CGGGCACCAATCCTTTCA-3'	(Viegas et al., 2017)[21]
Probe	5'-CGTCAATAAGCGCTTTT-3'	
Versicolores		
Forward Primer	5' – CGGCGGGGAGCCCT-3'	
Reverse Primer	5' – CCATTGTTGAAAGTTTTGACTGATcTTA-3'	(EPA, 2017)[36]
Probe	5' – AGACTGCATCACTCTCAGGCATGAAGTTCAG-3'	

Table 2 - Minimum, maximum, median and interquartile range for fungal and *Aspergillus* spp. load (MEA and DG18).

		Filter samples		
	Statistics	Forklifts	Taxis	Controls
Fungal load	Minimum	0,0	0,0	0,0
(MEA)	Maximum	40000,0	1500,0	68500,0
	Median	8000,0	0,0	500,0
	Interquartil range	14000,0	0,0	600,0
Fungal load	Minimum	0,0	0,0	0,0
(DG18)	Maximum	67000,0	6500,0	56000,0
	Median	15500,0	0,0	0,0
	Interquartil range	17500,0	500,0	500,0
Aspergillus spp. load	Minimum	0,0	0,0	0,0
(MEA)	Maximum	24500,0	0,0	500,0
	Median	4500,0	0,0	0,0
	Interquartil range	11000,0	0,0	0,0
Aspergillus spp. load	Minimum	0,0	0,0	0,0
(DG18)	Maximum	29500,0	1000,0	500,0
	Median	2500,0	0,0	0,0
	Interquartil range	15500,0	0,0	0,0

Table 3 - Prevalence rate of the fungal load in MEA and DG18.

		Filter samples							
		Forklifts		Taxis		Controls		Total	
		n	%	n	%	n	%	n	%
Fungal load	absence	1	5,9	14	77,8	17	45,9	32	44,4
(MEA)	presence	16	94,1	4	22,2	20	54,1	40	55,6
	Total	17	100,0	18	100,0	37	100,0	72	100,0
Fungal load	absence	1	5,9	11	61,1	20	54,1	32	44,4
(DG18)	presence	16	94,1	7	38,9	17	45,9	40	55,6
	Total	17	100,0	18	100,0	37	100,0	72	100,0

n – isolates number

Table 4 – Azole-resistant *Aspergillus* spp. distribution on filter samples

				Aspergillus spp. distribution in azole-supplemented media
	Filters samples	n	%	ID (n; %)
Aspergillus spp.load (ITC)	Forklifts	3450	76.7	*Nigri* (3418; 76.0), *Circumdati* (17; 0.4), Candidi (14; 0.3), *Aspergilli* (1; 0.02)
	Taxis	3	50.0	*Candidi*
	Controls	0	0	
	Total	3453	76.6	
Aspergillus spp.load (VRC)	Forklifts	2009	43.1	*Nigri*
	Taxis	0	0	
	Controls	0	0	

	Total	2009	37.4	
Aspergillus spp.load (PSC)	Forklifts	5	0.3	*Nigri*
	Taxis	0	0	
	Controls	0	0	
	Total	5	0.3	
Aspergillus spp. load (SAB)	Forklifts	3599	69.1	*Nigri* (3534; 67.8), *Fumigati* (57; 1.1), *Candidi* (7; 0.13), *Flavi* (1; 0.02)
	Taxis	0	0	
	Controls	0	0	
	Total	3599	62.4	

ITC, itraconazole; VRC, voriconazole; PSC, posaconazole; SAB, Saboraud.

n, number of *Aspergillus* species isolates;

%, number of *Aspergillus* species isolates per total of resistant isolates

Table 5 - Results of Kruskal-Wallis test to compare fungal and *Aspergillus* spp. load between the three samples groups

	Ranks		Test Statistics [a, b]			Multiple Comparisons
Filters samples	n	Mean Rank	Chi-Square	df	p	

Fungal load (MEA)	Forklifts	17	56,82				Forklifts ≠ Taxis (p=0.000)
	Taxis	18	22,61				Forklifts ≠ Controls (p=0.000)
				27,059	2	0.000*	
	Controls	37	33,92				
	Total	72					
Fungal load (DG18)	Forklifts	17	59,68				Forklifts ≠ Taxis (p=0.000)
	Taxis	18	27,28				Forklifts ≠ Controls (p=0.000)
				30,331	2	0.000*	
	Controls	37	30,34				
	Total	72					
Aspergillus spp.load (MEA)	Forklifts	17	62,15				Forklifts ≠ Taxis (p=0.000)
	Taxis	18	27,50				Forklifts ≠ Controls (p=0.000)
				57,960	2	0.000*	
	Controls	37	29,09				
	Total	72					
Aspergillus spp. load (DG18)	Forklifts	17	51,71				Forklifts ≠ Taxis (p=0.000)
	Taxis	18	34,81				Forklifts ≠ Controls (p=0.000)
				25,778	2	0.000*	
	Controls	37	30,34				
	Total	72					

a. Kruskal Wallis Test

b. Grouping Variable: Filters samples

*Statistical significance difference at 5% significance level

Figure 1– Aspergillus spp. prevalence on filters samples in MEA and DG18

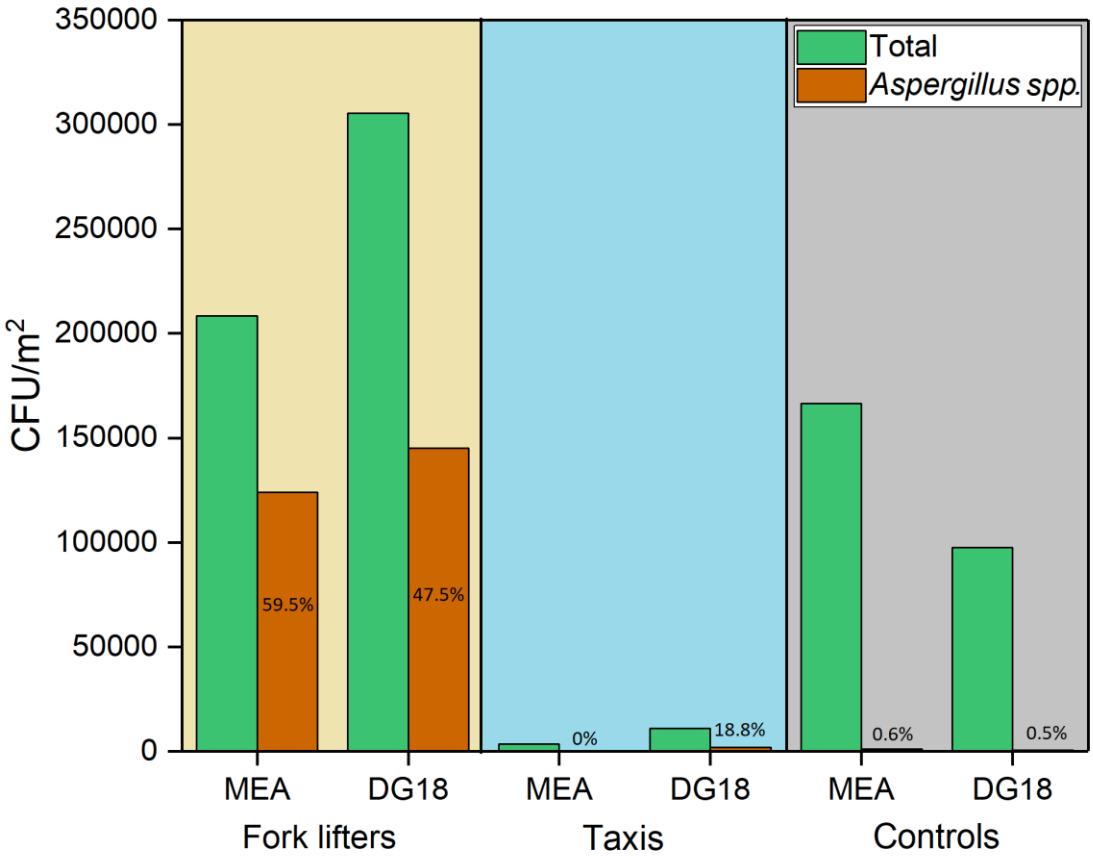

Figure 2 – Aspergillus spp. sections distribution on forklifts filter samples