atmosphere

MDPI

Article

Microbiological Contamination Assessment in Higher Education Institutes

Carla Viegas [1,2,3,*], Raquel Pimenta [1], Marta Dias [1], Bianca Gomes [1], Miguel Brito [1], Liliana Aranha Caetano [1,4], Elisabete Carolino [1] and Anita Quintal Gomes [1,5]

1 H&TRC-Health & Technology Research Center, ESTeSL-Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, 1990-096 Lisboa, Portugal; raquel.pimenta@estesl.ipl.pt (R.P.); marta.dias@estesl.ipl.pt (M.D.); bianca.gomes@estesl.ipl.pt (B.G.); miguel.brito@estesl.ipl.pt (M.B.); liliana.caetano@estesl.ipl.pt (L.A.C.); etcarolino@estesl.ipl.pt (E.C.); anita.gomes@estesl.ipl.pt (A.Q.G.)
2 Public Health Research Centre, NOVA National School of Public Health, Universidade NOVA de Lisboa, 1099-085 Lisboa, Portugal
3 Comprehensive Health Research Center (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, 1169-056 Lisboa, Portugal
4 Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, 1649-003 Lisboa, Portugal
5 Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal
* Correspondence: carla.viegas@estesl.ipl.pt

Abstract: The higher education sector represents a unique environment and it acts as a work environment, a learning environment for students, and frequently, also a home environment. The aim of this study was to determine the microbial contamination (*SARS-CoV-2*, fungi, and bacteria) in Higher Education Facilities (HEI) by using active and passive sampling methods and combining culture-based methods with molecular tools targeting *Aspergillus* section *Fumigati*. In addition, the resistance to azole profile was also assessed. Surface samples showed a range of total bacterial contamination between 1×10^3 to 3.1×10^6 CFU·m^{-2}, while Gram-negative bacteria ranged from 0 to 1.9×10^4 CFU·m^{-2}. Fungal contamination ranged from 2×10^3 to 1.8×10^5 CFU·m^{-2} on MEA, and from 5×10^3 to 1.7×10^5 CFU·m^{-2} on DG18. The most prevalent species found on both media was *Cladosporium* sp. (47.36% MEA; 32.33% DG18). *Aspergillus* genera was observed on MEA (3.21%) and DG18 (14.66%), but not in the supplemented media used for the azole screening. *Aspergillus* section *Fumigati* was detected in 2 air samples (2.22%, 2 out of 90 samples) by qPCR. When testing for *SARS-CoV-2* all results were negative. The present study showed that although cleaning and disinfection procedures are done regularly due to the COVID-19 pandemic, being effective in eliminating *SARS-CoV-2*, surfaces were often contaminated with microorganisms other than *SARS-CoV-2*. This can be a result of increasing resistance to biocides, and to the wide range of environmental factors that can contribute to the dissemination of microbial contamination indoors.

Keywords: active and passive sampling; fungi; bacteria; azole resistance screening; *Aspergillus* section *Fumigati*

check for updates

Citation: Viegas, C.; Pimenta, R.; Dias, M.; Gomes, B.; Brito, M.; Aranha Caetano, L.; Carolino, E.; Gomes, A.Q. Microbiological Contamination Assessment in Higher Education Institutes. *Atmosphere* **2021**, *12*, 1079. https://doi.org/10.3390/atmos12081079

Academic Editor: Daniele Contini

Received: 25 July 2021
Accepted: 19 August 2021
Published: 23 August 2021

1. Introduction

In the last few decades, several studies were conducted to understand the impact of indoor air quality (IAQ) in public health in different environments, including residential building, shopping malls, schools, health care centers, offices, museums, libraries, temples, and churches, among others [1–7]. It was indicated that decreased IAQ can negatively affect human health as most people spend around 90% of their time indoors, mainly at home or in the workplace [8]. Due to this, IAQ has emerged and received increasing attention from international scientific community, political institutions, and environmental governances [2,8,9]. The indoor air pollution (IAP), that refers to the existence of pollutants,

such as volatile organic compounds (VOCs), particulate matter (PM), inorganic compounds, physical chemicals, and microbiological agents, can influence negatively the IAQ and are harmful to the human health [8,9].

Health effects from indoor air pollutants may be experienced soon after exposure (immediately) or, possibly, years later (long-term) [10]. The respiratory system is often the primary to be affected by the IAP effects [8]. Exposure to microorganisms, in particular, is associated with irritating effects (eyes, nose, skin), allergic reactions (asthma, rhinitis), digestive problems, infectious diseases (pneumonia, tuberculosis, Legionnaire's disease, severe acute respiratory syndrome), and toxic reactions due to the presence of mycotoxins [8,11,12]. Furthermore, it is important to take into account that immunocompromised individuals are more susceptible to acquiring infections caused by microorganisms, which can sometimes be fatal [11,13–15]. A common example is that of *Aspergillus* conidia, which can be ubiquitous and are easily dispersed in the air [16]. From the various *Aspergillus* sections, *Fumigati* is the genus most frequently associated with respiratory symptoms [17]. Another major concern regarding microbial contamination is the emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance, making it difficult or impossible to treat common infections [18]. In fact, the development of resistance to antifungal drugs, particularly in section *Fumigati*, is a phenomenon with growing prevalence in Europe that has been associated with therapeutic failure and high mortality rates [19]. Furthermore, the co-prevalence of *SARS-CoV-2* in these environments can pose a serious threat to the health of all occupants [20]. In this context, assessing the microbial contamination is a useful and important tool of prevention to microbial exposure and to guarantee IAQ [21].

The higher education sector represents a unique environment acting as a work environment for faculty members, a learning environment for students, and frequently, a home environment for students [22]. Thus, faculties comprise a high population density that spend more than eight hours per day, five days a week inside their buildings [22–24]. In addition, the higher education buildings have a very wide usage as they include lecture halls, gyms, dormitories, day care, restaurants, garden, and laboratories [23,24]. Since many outbreaks of epidemic diseases are correlated with the presence of microorganisms in the air (for example, influenza A pandemic—H1N1 2009—was frequently reported in universities in 2009), the assessment of the level of microbiological contamination, especially in crowed places at universities, should be performed regularly [25].

Assessment of microbiological contamination in HEI has previously been performed in several facilities, such as classrooms, sports hall, laboratories, rooms, entrances, libraries, cafeterias, and restaurants [21,23,24,26–34]. However, each of the above-mentioned studies at universities focused mainly on a single type of indoor environment [35]. Importantly, *SARS-CoV-2* detection has been carried out in a very limited number of HEI [36], being very relevant to assess it in additional HEI and compare its levels with those of other microorganisms that might be present indoors.

The aim of this study was to determine the microbial contamination (*SARS-CoV-2*, fungi, and bacteria) in HEI environment by using active and passive sampling methods and combining culture-based methods and molecular tools targeting *Aspergillus* section *Fumigati*. In addition, the resistance to azole profile was also assessed in the HEI analyzed.

2. Materials and Methods

2.1. Facilities Assessed and Sampling Sites

This study was performed between July and December 2020 in ten selected facilities of Portuguese HEI, including the presidency building (PB), social services (SS) and eight faculties (HEI 1 to HEI 8). It was part of an enlarged cross-sectional study with financial support to implement an integrated approach on Occupational Health to tackle the COVID-19 pandemic and microbiological contamination assessment aiming to ensure safety in academic recovery during the pandemic crises [37].

The ten facilities under study are located in the Lisbon district and all presented different core activities depending of their purpose and graduation courses held (Table 1).

The sampling sites were chosen based on previous selection by areas of facility by the Occupational Health Services, in the scope of *SARS-CoV-2* surveillance held during the 2nd pandemic wave in Portugal and before starting the 2nd semester of the academic year 2020/2021. A walkthrough survey and checklist were applied in order to prioritize the most critical workplaces/areas in relation to *SARS-CoV-2* contamination. In summary, sampling sites of each facility were selected according to the following criteria: 50–80% workplaces/facilities occupation, activities performed that can lead to higher number of workers per workplace and/or without a mask (cantine) or based on workers positive serologic surveillance results (positive result for IgM+ or IgG+) [37]. All the facilities implemented a contingency plan that included working, whenever possible, in home office even concerning theoretical lectures, wearing a mask indoor and outdoor (when 2 meters distance was not possible to ensure) and workers should remain at home (quarantine) when COVID-19 symptoms arise until further diagnose.

Environmental samples (air and surface samples) were performed in each area and 6 to 25 samples were collected at each location (Table 1). Most of the sampling sites were common among all the facilities, such as offices (including human and financial resources, academic services, logistics, accounting, and acquisition department), attendance room, reception rooms, auditoriums, meals spaces, bathrooms and libraries. Although there are classrooms in almost every location, the type of classroom analyzed varied widely in all locations, including rooms for music, dance, choir, theatre and multimedia, laboratories, and gyms. In addition, in some locations, there were samples collected from student's social rooms, workshops, changing rooms and professors' room (Table 1).

Table 1. Developed activities, areas sampled and samples number in each facility.

Study Site	Develped Activities/Graduation Courses Held	Assessed Areas	Surface Swabs (for *SARS-CoV-2*)	Surface Swabs (for Fungi and Bacteria)	Air Samples
PB	Presidency services that support the HEI in the activities common to the institution	Communication Office (Doorknob)	1	1	0
		Communication Office (Ventilation grids)	1	1	
		Drivers Reception (Counter)	1	1	
		Entrance (Touch screen and money)	1	1	
		Entrance (Buttons)	1		
		Lunchroom (Microwave and Coffee machine)	1	0	
		Lunchroom (Refrigerator)	1	0	
		Lunchroom (Ventilation grids)	1	1	
		Human Resources (Coffee machines)	1	0	
		Human Resources (Access card)	1	0	
		Human Resources (Staplers and stamps)	1	0	
		Human Resources (Ventilation grids)	1	1	
		Financial Resources (Staplers and stamps)	1	1	
		Human and Financial Resources (Windows)	1	0	
		Human and Financial Resources (Doorknob)	1	0	
		Financial Resources (Ventilation grids in operation)	1	1	
		Reception 1 (Printer)	1	0	
		Reception 2 (Table)	1	0	
		Acquisition Department (1st floor) (Windows)	1	0	
		Acquisition Department (1st floor) (Keys and card)	1	0	
		Acquisition Department (1st floor) (Ventilation grids in operation)	1	1	
		Male Bathroom (1st floor) (Doorknob and Faucets)	1	0	
		Female Bathroom (1st floor) (Doorknob and Faucets)	1	0	
		Corridor (1st floor) (Printer)	1	0	
		Academics, Quality and Planning Department (Mobile air conditioning equipment)	1	0	

Table 1. *Cont.*

Study Site	Develped Activities/Graduation Courses Held	Assessed Areas	Surface Swabs (for *SARS-CoV-2*)	Surface Swabs (for Fungi and Bacteria)	Air Samples
SS	Social services that implement the school social action policy and the provision of support and benefits to students who attend the institution	Lunchroom (Table)	0	1	
		Lunchroom (Microwave, Refrigerator)	1	1	1
		Lunchroom (Door)	1	1	
		Waiting Room/Classroom (Coffee Machine)	1	0	
		Waiting Room/Classroom (Coffee Machine, PC and eraser)	0	1	1
		Waiting Room/Classroom (Table)	1	1	
		Reception (Counter)	1	1	
		Reception (Door and Water Machine)	1	1	1
		Bathroom (Doors and Faucets)	1	1	
		Bathroom (Floor)	0	1	1
		Attendance Room (Table and Acrylic Protection)	1	1	1
		Accounting (Printer)	1	1	
		Accounting (Doors)	1	0	1
		Accounting (Floor)	0	1	
HEI 1	Health sciences and technologies for the exercise of skills in the field of disease prevention and health promotion, diagnosis and therapeutic intervention and rehabilitation	Logistics (Door and Window)	1	1	1
		Accounting/Provisioning (Printer, Door and Stapler)	1	1	1
		Academic Services (Printer SA and Printer corridor)	1	1	1
		Reception (Door, Handrail and Balcony)	1	1	0
		Bar (Chairs and Door)	1	1	1
		Library (Printer and Computer)	1	1	1
		Lunchroom (1st floor) (Fridge and microwave)	1	1	1
		Lunchroom (2nd floor) (Fridge and microwave)	1	1	1
		Corridor (1st floor) (Printer)	1	1	0
		Bathroom (1st floor) (Doorknobs, Door and Flush toilet)	1	1	0
		Office (Mouse, Keyboard, Light switch, Door and Doorknob)	1	1	0
		Corridor (2nd floor) (Printer)	1	1	0
		Bathroom (2nd floor) (Doorknobs, Door and Flush toilet)	1	1	0
HEI 2	Accounting and business technicians to perform administrative and financial accounting activities of national organizations	Financial Services (Printers and Stapler)	1	1	1
		Professors Room 1 (Printers and Computers)	1	1	1
		Professors Room 2 (Printers, Computers and TV control)	1	1	1
		Cafeteria/Bar (Counter and Tables)	1	1	1
		Auditorium (Handrail, Light switch and Door)	1	1	1
		Student's Social Room (Door, Fridge, Microwave, Faucet, Coffee and Machine)	1	1	1
		Library (Printer and Computers)	1	1	1
		Human Resources (Printer, Door, Acrylics, Counter and Light switch)	1	1	1
HEI 3	Classic engineering areas and in the area of renewable energies, acoustics, environment, hygiene and safety, urban rehabilitation, biomedical engineering and management	Library (Computer, Printer and Doorknob)	1	1	1
		Lunchroom 1 (Faucet, Doorknob, Chair, Table, Microwave, Coffee machine and Faucet)	1	1	1
		Lunchroom 2 (Microwave, Toaster, Kettle, Doorknob, Door, Table and Chair)	1	1	1
		Bar 1 (Microwave, Refrigerator, Handles, Cash register and ATM)	1	1	1
		Workshop (Machine, Doorknob and Door)	1	1	1
		Bar 2 (Cash register, Control, Fridge and Microwave)	1	1	1
		Organic Chemistry Lab 1 (Scale and Faucets)	1	1	1
		Organic Chemistry Lab 2 (Equipments, Door and Doorknob)	1	1	1
		Inorganic Chemistry Lab (Computer, Doorknobs, Equipments, Micropipettes and Refrigerator)	1	1	1
		Canteen (Faucets, Counter, Acrylic and Cash Register)	1	1	1
		Auditorium (Doorknobs, Door, Table, Chair and Eraser)	1	1	1

Table 1. *Cont.*

Study Site	Develped Activities/Graduation Courses Held	Assessed Areas	Surface Swabs (for *SARS-CoV-2*)	Surface Swabs (for Fungi and Bacteria)	Air Samples
HEI 4	Theater and cinema, including conducting research activities, experimentation and artistic production	Library (PC, Windows and Tables)	1	1	1
		Canteen (Chairs, Table, PC and Doorknob)	1	1	1
		Bar (Counter, Napkin Holder, PC, Coffee Machine and Table)	1	1	1
		Grand Auditorium (Doorknob, PC and Chairs)	1	1	1
		Small Auditorium (Scenic Interpretation Studio) (Door, Table, Doorknob and Chairs)	1	1	1
		Computer Room (Switch, Mouse, PC, Keyboard, Door and Doorknob)	1	1	1
		Workshop (Doorknob, Tables, Lockers and Equipment)	1	1	1
HEI 5	Teachers, and other educational agents with a high level of cultural, scientific, technical, and professional preparation	Bar/Cafeteria (Cash Register and Counters)	1	1	1
		Science Laboratory (Physic-Chemical) (Chairs, Scales, Faucets and Handle)	1	1	1
		Student's Social Room (Microwave, Faucet, Windows and Chairs)	1	1	1
		Theatre and Choir Room (Switch, Door, Doorknob, Windows, Tables and Chairs)	1	1	1
		Changing room/Dressing room (Doors, Doorknobs, Faucets)	1	1	1
		Gym (Doors, Doorknobs, Table, Chair, Equipment)	1	1	1
		Ceramic Workshop (Tables, Chairs, Faucets, Eraser, Doorknob, Door)	1	1	1
		Dance Room (Doorknobs, Doors, Eraser)	1	1	1
		Music Room (Switch, Piano, Doorknob, Window and Chairs)	1	1	1
HEI 6	Artistic, technical, technological, and scientific training for music professionals	Grand Auditorium (Chairs, Tables, Piano and Tripods)	1	1	0
		Small Auditorium (Chairs, Tables, Piano and Tripods)	1	1	
		Library (Computers and Tables)	1	1	
		Academic Services (Printer, Doorknob and Cabinets)	1	1	
		Corridor (Printer)	1	1	
		Academic Services (Customer Service) (Acrylic, Pens, Coffee Machine and Tables)	1	1	
		Lunchroom (Microwave, Tables, Chairs, Refrigerator, Doorknobs and Switch)	1	1	
		Choir Room (Chairs, Tables, Piano, Eraser, Switch, Door and Doorknobs)	1	1	
		Music Room (Eraser, Tripods, Acrylic, Door, Doorknob, Switch and Piano)	1	1	
HEI 7	Social communication, including the areas of audiovisual and multimedia, journalism, advertising and marketing, and public relations and business communication	Bar (Chairs, Tables, Cash Register, Acrylic, Counter and Calculator)	1	1	0
		Study Room (Food and coffee machines, Chairs and Tables)	1	1	
		Corridor (Printer)	1	1	
		"Home Food" Space (Microwave, Water machine, Tables and Chairs)	1	1	
		Auditorium (Door, Doorknob and Chairs)	1	1	
		Academic Services (Tables, Switch, Chairs, Acrylic, Printer, Stapler, Hole Puncher, Door and Doorknob)	1	1	
		Library (Tables, Chairs and Acrylic)	1	1	
		Multimedia Warehouse (Printer, Computers, Keyboards, Mouse, Barcode reader, Microwave, TV and Chairs)	1	1	

Table 1. *Cont.*

Study Site	Develped Activities/Graduation Courses Held	Assessed Areas	Surface Swabs (for *SARS-CoV-2*)	Surface Swabs (for Fungi and Bacteria)	Air Samples
HEI 8	Dance	Academic Services (Printer, Doorknob, Acrylic and Table)	1	1	0
		Dance Reception (Doorknob, Computer, Telephone and Keys)	1	1	
		Professors Room (Doorknob, Printer, Switch, Flush toilet, Table and Chairs)	1	1	
		Atrium Dance Studio (Bar and Sound System)	1	1	
		Dance Studio 1 (Bar and Sound System)	1	1	
		Dance Studio 2 (Bar and Sound System)	1	1	
Total			106	92	48

The samples were collected mainly in the morning and during normal activities, except for HEI 1, where the samples were collected in two days, one of them in the afternoon. In all facilities, the cleaning method applied was based on cleaning and disinfection recurring to bactericide and virucide, bleach and multipurpose detergent. Most of the sampling sites (56.8%) registered between 1 to 9 workers to follow the contingency plans. However, the accurate number of workers was not possible to obtain due to workers quarantine in the same day of the assessments to comply with contingency plans.

2.2. Samples Collected and Assays Performed

Air samples of 600 L were collected using the impinger Coriolis µ air sampler (Bertin Technologies, Montigny-le-Bretonneux, France) with a flow rate of 300 L/min collected into a conical vial containing 5 mL Buffer NVL (NZY Viral RNA Isolation kit (MB40701) component) (Figure 1). Surface samples were collected by swabbing the areas of each sampling site, using sterile cotton swabs moistened in Buffer NVL (*SARS-CoV-2* assessment) or sterilized water (fungi and bacteria assessment). A 10 cm × 10 cm square stencil, disinfected between samplings with a 70% alcohol solution was used (ISO 18593: 2004) to allow quantification. On some surfaces with common characteristics, such as surfaces material and cleaning procedures, composite samples were performed (swabbing different surfaces with the same swab) [38] (Figure 1).

Culture based methods were applied only in surface samples. Every swab was later extracted with 1 ml of 0.1% Tween™ 80 saline solution (NaCl 0.9%) for 30 min at 250 rpm on an orbital laboratory shaker (Edmund Bühler SM-30, Hechingen, Germany) and plated onto the selected media. Four different culture media were used in order to enhance the selectivity for bacterial and fungal growth, as follows: 2% malt extract agar (MEA) with 0.05 g L−1 chloramphenicol media, and dichloran glycerol (DG18) agar based media, for fungal characterization; Tryptic Soy Agar (TSA) with 0.2% nystatin, for total bacteria assessment; and Violet Red Bile Agar (VRBA), for Gram-negative bacteria.

Antifungal resistance was also screened by inoculating 150 µL of the samples on Sabouraud dextrose agar (SDA) supplemented with 4 mg/L itraconazole (ITR), 2 mg/L voriconazole (VOR), 0.5 mg/L Posaconazole (POS), or SDA alone (as control) (adapted from the EUCAST guidelines [39]).

All the inoculated plates were incubated at 27 °C for five days for fungal growth (four days regarding azole resistance screening) or for seven days at 30 °C and 37 °C for bacterial growth and for Gram-negative bacterial growth, respectively. After the incubation period, quantitative (colony-forming units—CFU·m^{-2}) results for fungi and bacteria were obtained. When colony overgrowth was observed due to fungi with fast growing rates (Mucorales, *Chrysonilia sitophila* and *Trichoderma* sp.), making it impossible to count colonies, the median of all colony values obtained in all locations of the same facility was assumed. Fungal species were also identified microscopically using lactophenol cotton blue mount

procedures. Morphological identification was achieved through macro and microscopic characteristics [40].

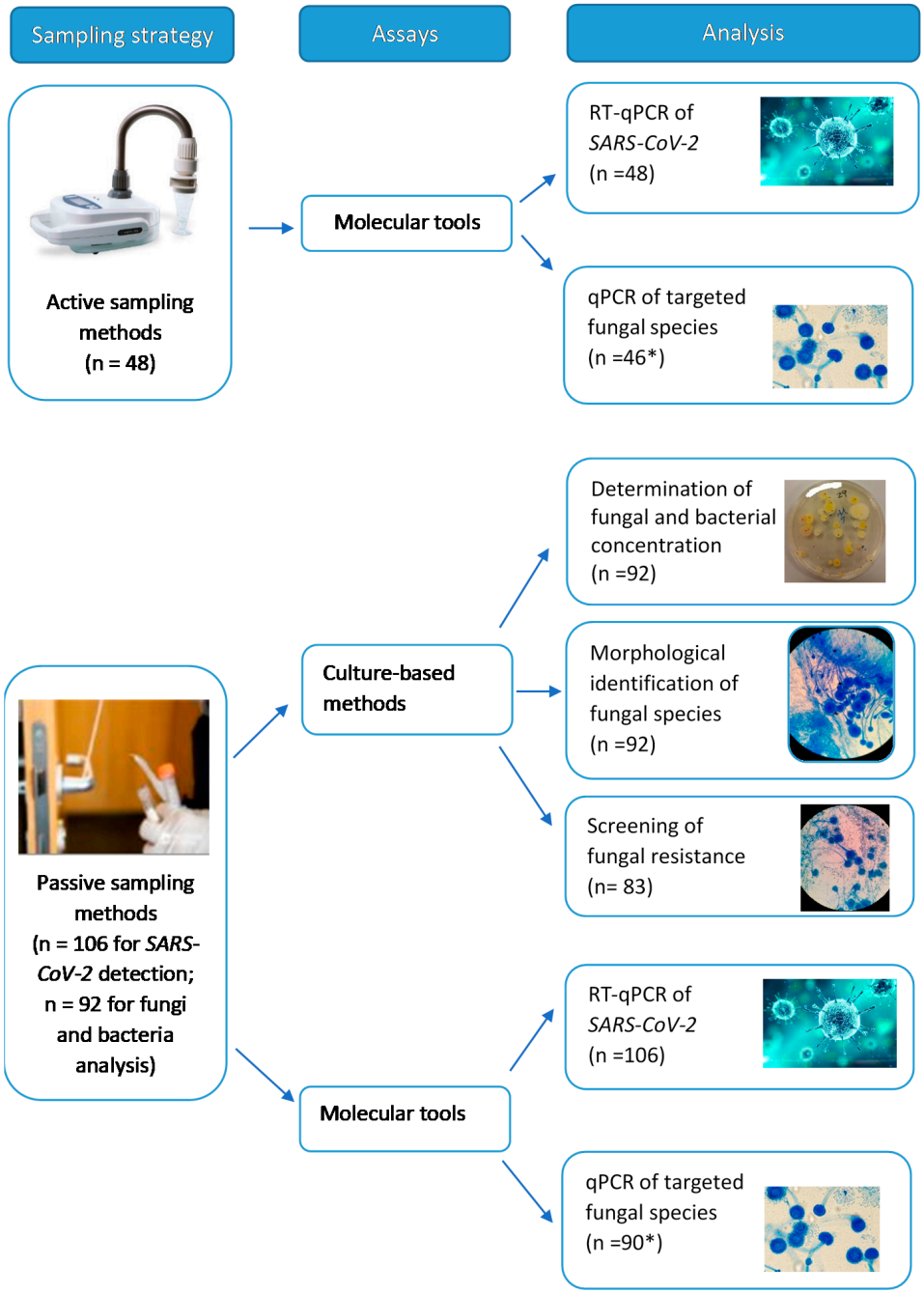


Figure 1. Sampling strategy adopted and assays applied. * Lack of extracts quantities in two samples to perform the assay.

Air samples obtained by impinger were also analyzed by molecular detection of *Aspergillus* section *Fumigati*. Procedures and reactions were performed as previously reported [41].

Concerning *SARS-CoV-2* assessment on air and surfaces, the procedures used for sample inactivation, RNA extraction and detection were as previously submitted [37]. In each analysis, a positive (a *SARS-CoV-2* positive sample) and a negative (water) sample were included. Moreover, in order to detect possible PCR inhibitors, an internal control was added to each PCR reaction (TATAA Universal RNA Spike I).

2.3. Statistical Analysis

Data were analyzed using SPSS statistical software, V26.0 for windows. The results were considered significant at the 5% significance level. To test the normality of the data, the Kolmogorov–Smirnov test was used. To characterize the sample, frequency analysis (n, %) was used for qualitative data. To study the relationship between bacterial and fungal counts and resistance to azoles and *Aspergillus* section, Spearman's correlation coefficient was used, since the assumption of normality was not verified.

3. Results

3.1. Viable Microbial Contamination

Surface samples showed a range of total bacterial contamination between 1×10^3 (classroom and attendance room) and 3.1×10^6 CFU·m^{-2} (offices). The highest median value (or total values in the case of classroom, attendance room, computer room, changing and dressing room, gym, study room and multimedia) was 5×10^5 CFU·m^{-2} (study room) and the lowest was 1×10^3 CFU·m^{-2} (classroom and attendance room). Gram-negative bacteria in surface samples ranged from 0 to 1.9×10^4 CFU·m^{-2} (laboratory) with a highest median value of 9.5×10^3 CFU·m^{-2} in the laboratory (Figures 2 and 3).

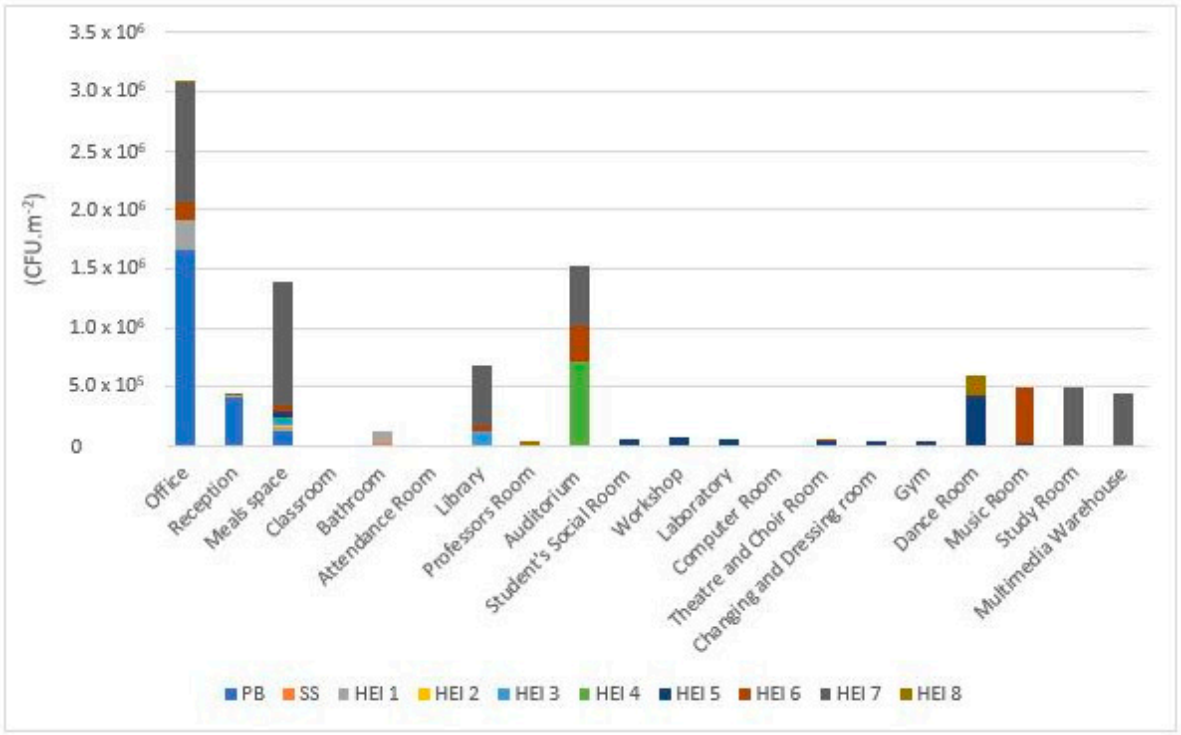


Figure 2. Total bacteria in surface swabs.

Total bacterial contamination found in different facilities ranged from 3.8×10^4 (HEI 2) to 4×10^6 CFU·m^{-2} (HEI 7), with the highest median value found in HEI 7 (5×10^5 CFU·m^{-2}) and the lowest median value found in Social Services (2×10^3 CFU·m^{-2}). Gram-negative bacteria ranged between 0 to 3×10^4 CFU·m^{-2} (HEI 3), with a highest median value of 4×10^3 CFU·m^{-2} (HEI 3) (Figures 2 and 3).

Fungal contamination on surfaces ranged from 0 to 1.5×10^5 CFU·m^{-2} (offices) on MEA, and from 0 to 1.8×10^5 CFU·m^{-2} (professors room) on DG18. The median values (or total values in the case of classroom, attendance room, computer room, changing and dressing room, gym, study room and multimedia) ranged from 0 to 5.9×10^4 CFU·m^{-2} (changing and dressing rooms) in MEA, and from 0 to 9.1×10^4 CFU·m^{-2} (professors room) in DG18 (Figures 4 and 5).

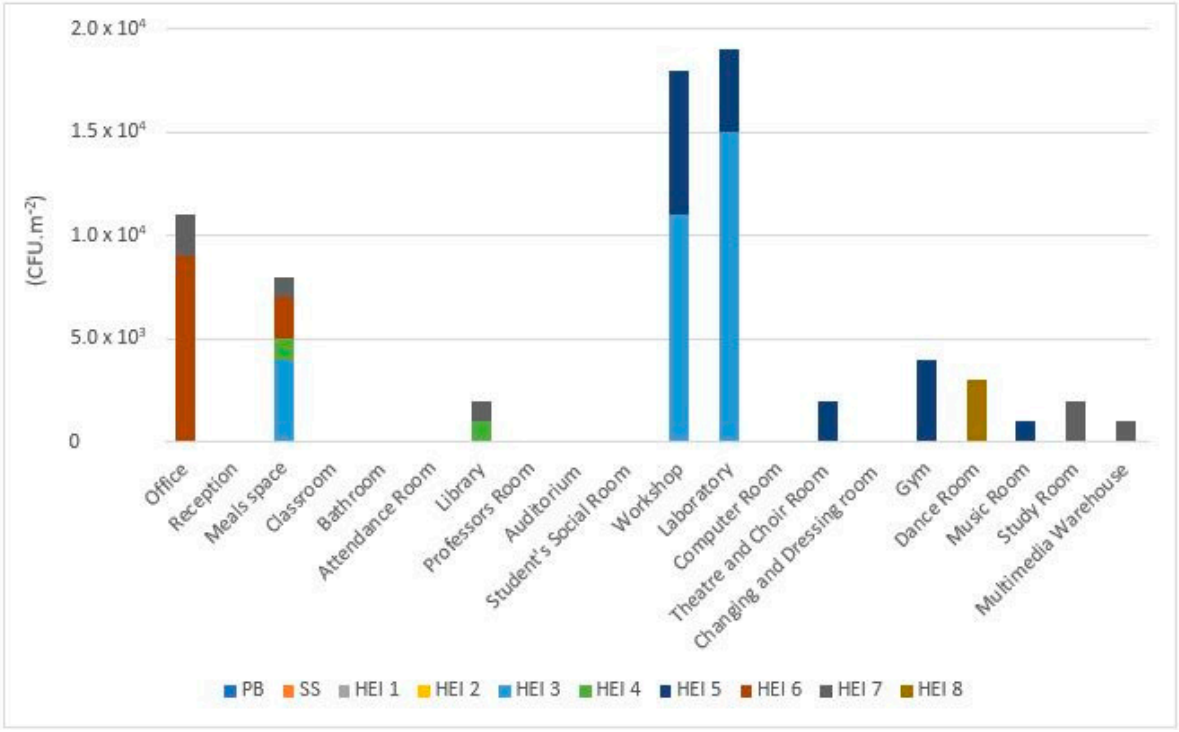


Figure 3. Gram-negative bacteria in surface swabs.

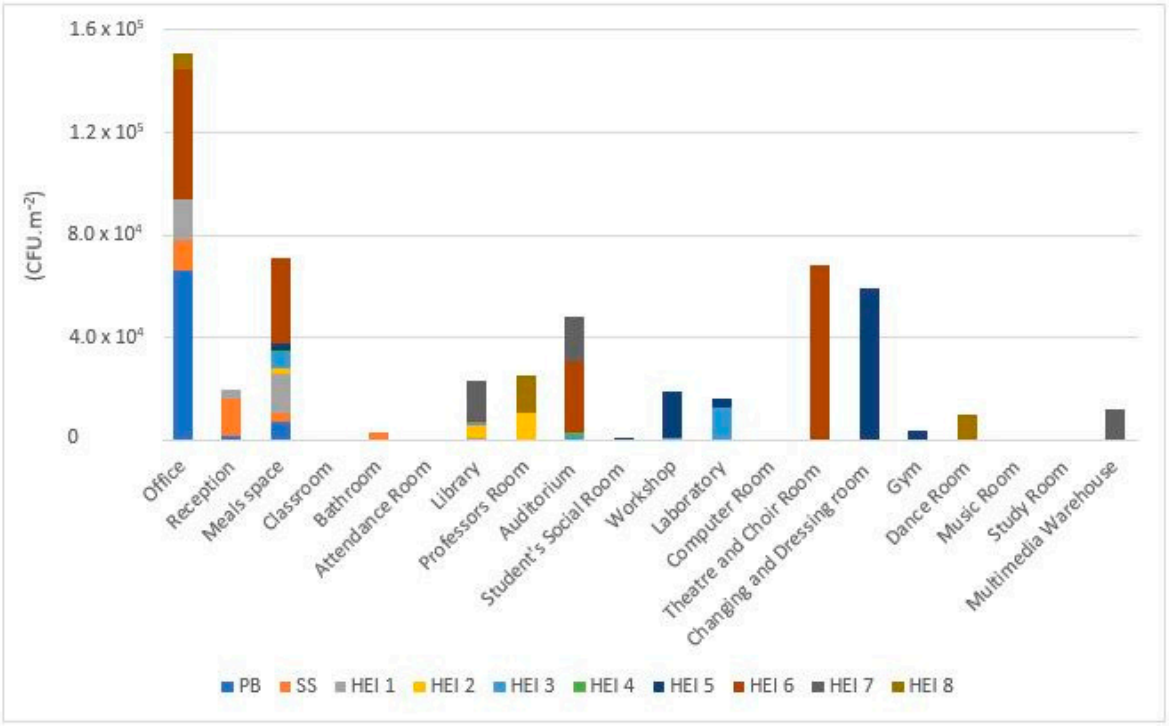


Figure 4. Fungal contamination in surface swabs on MEA.

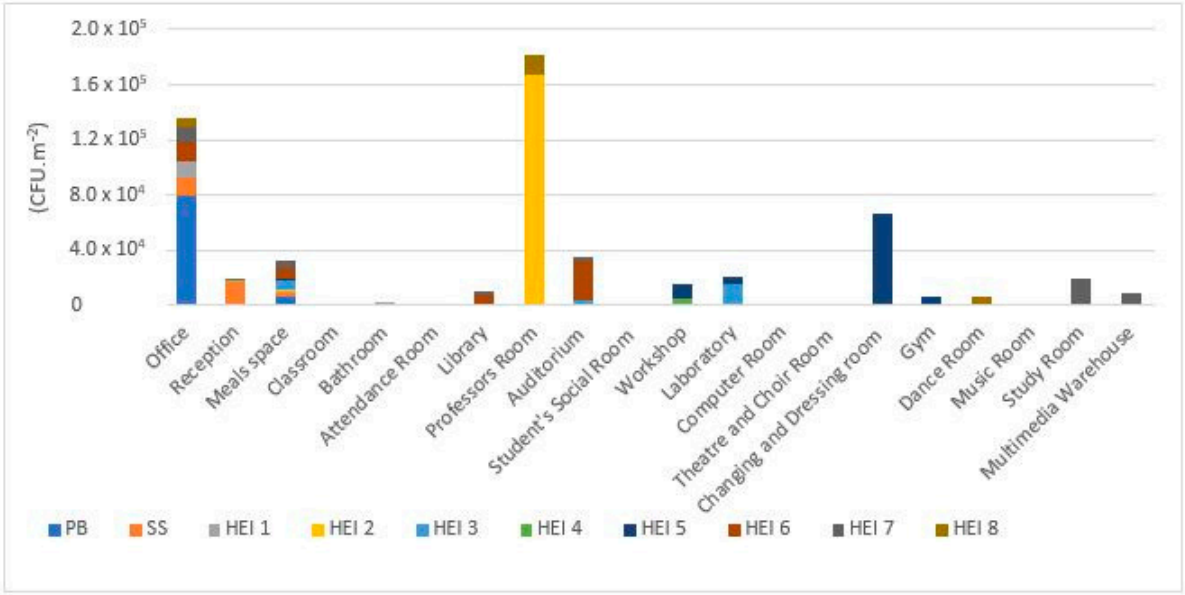


Figure 5. Fungal contamination in surface swabs on DG18.

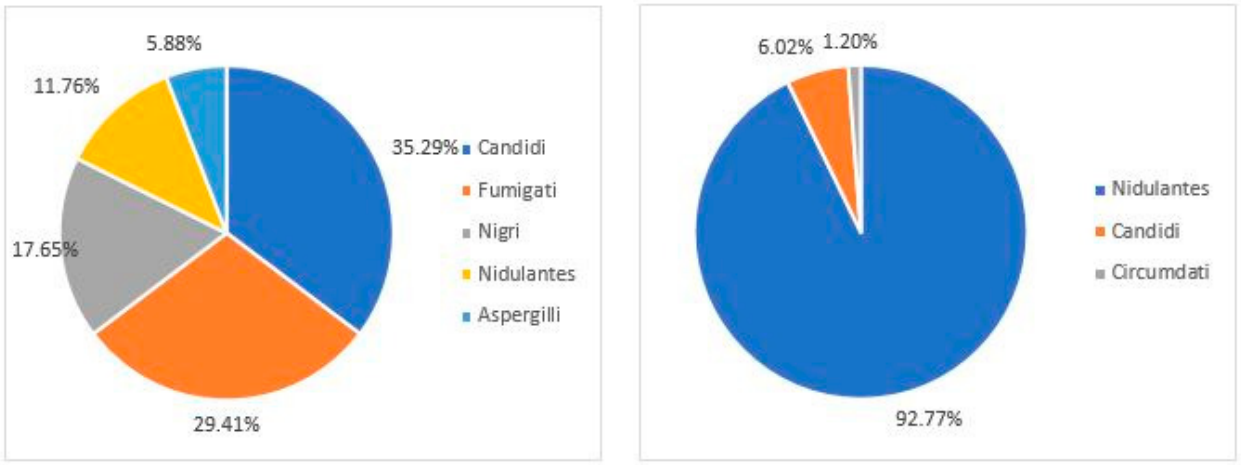
Fungal contamination found in different facilities ranged from 2×10^3 (HEI 4) to 1.8×10^5 CFU·m^{-2} (HEI 6) on MEA, and from 5×10^3 (HEI 4) to 1.7×10^5 CFU·m^{-2} (HEI 2) on DG18. The median values on MEA ranged between 0 (HEI 4) to 3.1×10^4 CFU·m^{-2} (HEI 6), and from 0 (HEI 4) to 8×10^3 CFU·m^{-2} (HEI 6) on DG18 (Figures 4 and 5).

Regarding fungal distribution 10 and 9 different fungal species were observed, on MEA and DG18, respectively. The most prevalent genera found on both media was *Cladosporium* sp. (47.36% MEA; 32.33% DG18), followed by *Penicillium* sp. (40.94%) and *Aspergillus* sp. (3.21%) on MEA, whereas on DG18 were *Aureobasidium* sp. (28.97%) and *Penicillium* sp. (20.67%).

Aspergillus genera was observed on MEA (3.21%) and DG18 (14.66%) (Table 2). There were found 5 different *Aspergillus* sections on MEA, being *Candidi* the most prevalent (6×10^3 CFU·m^{-2}; 35.29%), followed by *Fumigati* (5×10^3 CFU·m^{-2}; 29.41%), *Nigri* (3×10^3 CFU·m^{-2}; 17.65%), *Nidulantes* (2×10^3 CFU·m^{-2}; 11.76%) and *Aspergilli* (1×10^3 CFU·m^{-2}; 5.88%). On DG18 there were found 3 different *Aspergillus* sections, being *Nidulantes* the most identified (7.7×10^4 CFU·m^{-2}; 92.77%) followed by *Candidi* (5×10^3 CFU·m^{-2}; 6.02%) and *Circumdati* (1×10^3 CFU·m^{-2}; 1.20%) (Figure 6).

Table 2. Fungal species distribution in surface swabs after inoculation onto MEA and DG18 media.

MEA		DG18	
Fungi	n (CFU·m^{-2}); %	Fungi	n (CFU·m^{-2}); %
Cladosporium sp.	2.51×10^5; 47.36	*Cladosporium* sp.	1.83×10^5; 32.33
Penicillium sp.	2.17×10^5; 40.94	*Aureobasidium* sp.	1.64×10^5; 28.98
Aspergillus sp.	1.70×10^4; 3.21	*Penicillium* sp.	1.17×10^5; 20.67
Rhizopus sp.	1.40×10^4; 2.64	*Aspergillus* sp.	8.30×10^4; 14.66
Aureobasidium sp.	1.20×10^4; 2.26	*Chrysosporium* sp.	1.10×10^4; 1.94
Alternaria sp.	8.00×10^3; 1.51	*Fusarium* sp.	3.00×10^3; 0.53
Chrysosporium sp.	5.00×10^3; 0.94	*Chrysonilia* sp.	2.00×10^3; 0.35
Phoma sp.	3.00×10^3; 0.57	*Mucor* sp.	2.00×10^3; 0.35
Acremonium sp.	2.00×10^3; 0.38	*Acremonium* sp.	1.00×10^3; 0.18
Chrysonilia sitophila	1.00×10^3; 0.19		
Total	5.30×10^5; 100		5.66×10^5; 100



(**a**) (**b**)

Figure 6. *Aspergillus* sections distribution in surface swabs after inoculation onto (**a**) MEA and (**b**) DG18.

3.2. Azole Resistance Screening

Positive fungal growth on at least one azole supplemented media was observed for 10 fungal species. *Cladosporium* sp. was the most abundant species in all three azoles (64.71% on ITR; 65.49% on VOR; 53.33% on POS). *Penicillium* sp. was also found in all three azoles, being the second most prevalent species in two of three azoles (14.79% on VOR; 22.22% on POS). *Chrysosporium* sp., *Chrysonilia sitophila* and *Mucor* sp. were also presented in all three azoles. *Alternaria* sp. and *Aureobasidium* sp. were only observed on ITR and VOR. *Acremonium* sp. and *Rhizopus* sp. were only detected on VOR and POS, respectively (Table 3).

Table 3. Fungal distribution in azole-supplemented SAB media from surface swab samples.

	SAB	**ITR**	**VOR**	**POS**
Fungi	n (CFU·m^{-2}); %	n (CFU·m^{-2}); %	n (CFU·m^{-2}); %	n (CFU·m^{-2}); %
Acremonium sp.	1.10×10^4; 3.44	0.00	1.00×10^3; 0.70	0.00
Alternaria sp.	1.00×10^3; 0.31	2.00×10^3; 3.92	1.00×10^3; 0.70	0.00
Aspergillus section *Candidi*	2.00×10^3; 0.63	0.00	0.00	0.00
Aspergillus section *Fumigati*	3.00×10^3; 0.94	0.00	0.00	0.00
Aspergillus section *Nidulantes*	5.00×10^3; 1.56	0.00	0.00	0.00
Aspergillus section *Nigri*	8.00×10^3; 2.50	0.00	1.00×10^3; 0.70	0.00
Bipolaris sp.	3.00×10^3; 0.94	0.00	0.00	0.00
Aspergillus section *Circumdati*	1.00×10^3; 0.31	0.00	0.00	0.00
Aureobasidium sp.	9.00×10^3; 2.81	0.00	6.00×10^3; 4.23	0.00
Chrysonilia sitophila	1.00×10^3; 0.31	3.00×10^3; 5.88	4.00×10^3; 2.82	1.00×10^3; 2.22
Chrysosporium sp.	2.20×10^4; 6.88	9.00×10^3; 17.65	1.30×10^4; 9.15	8.00×10^3; 17.78
Cladosporium sp.	1.93×10^5; 60.31	3.30×10^4; 64.71	9.30×10^4; 65.49	2.40×10^4; 53.33
Mucor sp.	1.30×10^4; 4.06	2.00×10^3; 3.92	2.00×10^3; 1.41	1.00×10^3; 2.22
Penicillium sp.	4.40×10^4; 13.75	2.00×10^3; 3.92	2.10×10^4; 14.79	1.00×10^4; 22.22
Rhizopus sp.	3.00×10^3; 0.94	0.00	0.00	1.00×10^3; 2.22
Ulocladium sp.	1.00×10^3; 0.31	0.00	0.00	0.00
Total	3.20×10^5; 100	5.10×10^4; 100	1.42×10^5; 100	4.50×10^4; 100

3.3. Molecular Detection

Regarding *SARS-CoV-2*, all the environmental samples preformed presented negative results. *Aspergillus* section *Fumigati* was detected in 2 samples (2.22%, 2 out of 90 samples) (Table 4).

Table 4. Molecular detection of *Aspergillus* section *Fumigati*.

Sample Type	CFU·m^{-2} (in MEA/DG18)	C_q
Swab	0/0	31.56
	0/0	28.32

3.4. Correlation Analysis

Regarding bacterial counts in TSA, significant correlations were detected with counts in VRBA (rS = 0.252, p = 0.015), in SAB (rS = 0.354, p = 0.001), in VOR (rS = 0.235, p = 0.033) and in POS (rS = 0.343, p = 0.001) and with the number of workers (rS = 0.287, p = 0.009). These results indicate that higher bacterial counts in TSA are related to higher bacterial counts in VRBA, higher azole resistance counts (either in SAB, VOR or POS) and higher number of workers (Table 5).

Table 5. Study of the relationship between bacterial and fungal counts, azole resistance (SAB, ITR, VOR and POS) and *Aspergillus* sections (MEA, DG18) and azole resistance screening (SAB, VOR). Spearman correlation coefficient results.

		Bacterial Counts (CFU·m^{-2})	Fungal Counts (CFU·m^{-2})		Azole Resistance (CFU·m^{-2})				*Aspergillus* sp.				Number of Workers
									Fungi		Azole Resistance		
		VRBA	MEA	DG18	SAB	ITR	VOR	POS	MEA	DG18	SAB	VOR	
Bacterial counts	TSA	0.252 *	0.149	0.141	0.354 **	0.130	0.235 *	0.343 **	−0.,115	0.043	0.061	−0.009	0.287 **
	VRBA		−0.008	0.235 *	0.059	0.119	0.108	0.064	−0.098	−0.022	−0.061	−0.061	−0.082
Fungal counts	MEA			0.586 **	0.494 **	0.362 **	0.485 **	0.123	0.265 *	0.169	0.129	0.110	0.226 *
	DG18				0.562 **	0.479 **	0.572 **	0.314 **	0.001	0.321 **	0.140	0.002	0.084
Azole resistance	SAB					0.478 **	0.638 **	0.289 **	0.079	−0.023	0.388 **	0.070	0.182
	ITR						0.472 **	0.360 **	0.021	0.041	0.162	−0.058	−0.112
	VOR							0.308 **	0.243 *	0.090	0.375 **	0.133	0.171
	POS								−0.182	0.163	0.079	−0.062	−0.132
Aspergillus sp.	Fungi MEA									0.068	0.010	0.360 **	0.189
	Fungi DG18										−0.083	−0.025	−0.027
	Azole resistance SAB											0.29 4 **	−0.072
	Azole resistance VOR												0.190

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

Considering bacterial counts in VRBA, only significant correlation was found with fungal counts in DG18 (rS = 0.235, p = 0.024), revealing that higher bacterial counts in VRBA are related to higher fungal counts in DG18 (Table 5).

With regard to fungal counts in MEA, significant correlations were detected with fungal counts in DG18 (rS = 0.586, p = 0.000), in SAB (rS = 0.494, p = 0.000), in ITR (rS = 0.362, p = 0.001) and in VOR (rS = 0.485, p = 0.000), with *Aspergillus* sp. counts, fungi in MEA (rS = 0.265, p = 0.011) and with number of workers (rS = 0.226 p = 0.043). These results reveal that higher fungal counts in MEA are related to higher fungal counts in DG18, higher azole resistance (either in SAB, ITR or VOR), higher values of *Aspergillus* sp. counts in MEA and higher number of workers (Table 5).

With respect to fungal counts in DG18, significant correlations were detected with azole resistance in SAB (rS = 0.562, p =0.000), in ITR (rS = 0.479, p = 0.000), in VOR (rS = 0.572, p = 0.000) and in POS (rS = 0.314, p = 0.004) and with *Aspergillus* sp. counts in DG18 (rS= 0.321, p = 0.002). These results indicate that higher fungal counts in DG18 are related to higher azole resistance (either in SAB, ITR, VOR or POS) and higher values of *Aspergillus* sp. counts in DG18 (Table 5).

Regarding azole resistance in SAB, significant correlations were detected with azole resistance in ITR (rS = 0.478, p = 0.000), in VOR (rS = 0.638, p = 0.000) and in POS (rS= 0.289, p = 0.008) and with *Aspergillus* sp. counts—azole resistance in SAB (rS = 0.388, p = 0.000),

showing that higher counts in SAB are related to greater resistance to azoles in other media, including in the *Aspergillus* sp. counts (Table 5).

Concerning azole resistance in ITR, significant correlations were detected with azole resistance in VOR (rS = 0.472, $p = 0.000$) and in POS (rS = 0.360, $p = 0.001$), revealing that higher azole resistance in ITR is related with higher azole resistance in VOR and POS (Table 5).

With regard to azole resistance in VOR, significant correlations were detected with azole resistance in POS (rS = 0.308, $p = 0.005$) and with *Aspergillus* sp. counts in MEA (rS= 0.243, $p = 0.027$) and *Aspergillus* sp. counts in SAB (rS = 0.375, $p = 0.000$), revealing that higher azole resistance in VOR is related with higher azole resistance in POS, higher values of *Aspergillus* sp. counts in MEA and higher and *Aspergillus* sp. counts in SAB (Table 5).

Regarding *Aspergillus* section, the following significant correlations were found: (i) fungi in MEA and azole resistance in VOR (rS = 0.360, p = 0.001), which indicates that higher values in MEA are related to higher resistance to azoles in VOR; (ii) higher counts in SAB and VOR (rS = 0.294, $p = 0.007$), which reveals that higher counts in SAB is related to greater azole resistance in VOR (Table 5).

4. Discussion

The IAQ in HEI is of great importance due to the impact it has on the health and performance of students, professors and staff [22–24]. Furthermore, microbiological contamination assessment is one of the main parameters that affect IAQ, since potentially pathogenic microorganisms can be disseminated as bioaerosols and via contact with contaminated surfaces [8,9,42] or through resuspension from air to surfaces [42].

It has already been previously reported that the presence of fungi and bacteria in indoor air is influenced by a wide range of factors, such as human occupancy and their activities, humidity levels, ventilation, environmental characteristics, water infiltrations, construction and decoration materials and outdoor air [11,43,44]. Due to the extensive list of factors that influence IAQ, exposure assessment to microorganisms remains a challenge to every exposure assessor/industrial hygienist [45]. In fact, as in other risk factors exposure assessment studies, the sampling approach is of critical importance to achieve an accurate risk characterization regarding microbiological agents [46]. Active methods, based on air sampling, rely within the most common methods used for samples collection. However, they may not represent the real scenario regarding the inhalation exposure, since they can only reflect the load from a short period of time (mostly minutes), corresponding to the sampling duration, thus representing only a small fraction of the microbiological contamination exposure [47–49]. Despite these concerns, the impingement method, also based on air sampling, has been the most used for the *SARS-CoV-2* assessment in indoor environments [37], since it allows for longer active sampling times, thus ensuring collection of sufficient airborne viruses for detection by molecular tools [50].

In the case of passive methods, such as the surface swabs used in this study, they allow to characterize the contamination over a longer period of time (after the last cleaning procedure), thus providing a more comprehensive picture of the real exposure [46–49]. Indeed, they have previously been used in several indoor environments [46,51–61] generally providing more detailed and complete information regarding fungal species distribution. The use of both sampling methods allows to overcome each method limitation, ensuring a more precise exposure assessment [44]. This is further reinforced with the use of culture based-methods and molecular tools. Indeed, although most of the studies performed in HEI are focused on air quality screenings [28,30,32,33,62,63], surface analyses have also been shown to be relevant, as they may also reflect the contamination in the air by resuspension depending of the activities developed indoors, thus possibly leading to increased levels in airborne concentration [57,64,65].

Previous studies have shown that, besides the sampling approach, culture media applied also influence the results obtained for fungi and bacteria detection in environments [46,56]. Regarding bacterial contamination, culture media allows for the discrimi-

nation between total bacteria and Gram-negative bacteria [56]. As expected, in our study, the contamination of Gram-negative bacteria was lower than that of total bacteria, as expected, since the latter represents the number of Gram-positive bacteria and Gram-negative bacteria. The presence of bacteria on surfaces is a common situation, especially in the most frequently touched surfaces, as their main contamination sources are the occupants and their activities [66–68]. Thus, it was not surprising that the highest total bacterial counts were found in the offices (31.8%), followed by the auditorium (15.7%) and the meals space (14.4%), which are the places with higher occupancy. In fact, the positive correlation found between higher bacterial counts in TSA and higher number of workers emphasizes this contribution.

Although bacteria are ubiquitous and generally of human origin (from skin and mucous membranes) and not harmful for health, the presence of Gram-negative bacteria is a special concern, as they may have natural resistance to antibiotics and can also produce endotoxins, which can cause respiratory symptoms [11,43,57,68].

Regarding the fungal contamination assessment, besides the use of MEA, as suggested in the Portuguese guidance for IAQ assessment [11], DG18 was also selected to be used, since this media constitutes a better alternative for colony counting, also allowing to obtain higher diversity of genera [47,69]. Contrary to these expectations in this study we have obtained more diversification of fungal species in MEA (10 different species) than in DG18 (9 species) with the same trend being observed for *Aspergillus* sections (5 on MEA; 3 on DG18).

Interestingly, in the present study, the concentration of fungi on the surfaces was lower than that of bacteria, similarly to a study on surface swabs in university facilities [23]. The most prevalent fungi found in our study were *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp., which is in accordance with other studies based on the use of surfaces swabs as sampling approach [64,70–72].

While offices (28.5%), meals space (13.4%) and theatre and choir room (12.8%) were the areas most contaminated by fungi on MEA, on DG18 we detected higher fungal loads in professors' rooms (32.0%), offices (24.0%) and changing and dressing room (11.8%). A possible reason for the higher counts of fungi in these areas could be the fact that, due to the COVID-19 pandemic, all occupants were encouraged to open windows to prevent COVID-19 infection [20]. Indeed, evidence clearly indicated that opening windows increases the levels of fungi and other microorganisms in the air and on the surfaces as a result of the passage of outdoor air into indoors [33,42,67,73].

HEI 6 and HEI 2 have the highest counts of total fungal contamination on MEA and DG18 media, respectively. HEI 2 samples were analyzed in DG18 and are among the areas with higher contamination values, possessing a considerable concentration of fungi. However, in this building only professor's rooms were analyzed by this method. Fungal growth can be promoted in the presence of moisture, and many fungi grow easily on any surface that becomes wet or moistened, such as faucets, which are present, for example, in meals space and changing and dressing room [67,74,75].

Aspergillus sections, being most of them reported as mycotoxins producers (*Circumdati*, *Nigri*, *Fumigati*) [76], some with clinical relevance (*Fumigati*, *Nigri* and *Aspergilli*) [44,47,77], presented higher diversity on MEA media. Of note, while these sections were only identified in MEA, *Aspergillus* section *Circumdati* was only isolated on DG18 media, as previously reported [46]. These *Aspergillus* sections are considered indicators of harmful fungal contamination [11,77–79] and measurements should be applied to avoid their presence indoors. Furthermore, the detection of *Aspergillus* sections *Candidi* and *Nidulantes* should be emphasized due to their toxigenic potential [47,76,78].

The emergence of pathogenic fungi resistant to antifungal agents widely used in the treatment of fungal infections, which can cause therapeutic failure, has been notorious in recent years [80,81]. In this study, the screening of fungal resistance to three medical azoles was conducted. *Cladosporium* sp. was the fungal species mostly present in all three azoles (64.71% on ITR; 65.49% on VOR; 53.33% on POS), followed by *Penicillium* sp. Regarding

Mucor sp. and *Rhizopus* sp., they are intrinsically resistant to voriconazole, with itraconazole and posaconazole as first-line therapy [44,82]. However, in this study, we have observed the growth of these fungal species in the presence of itraconazole and posaconazole thus indicating the need to further characterize fungal resistance of those isolates [44,82].

The identification of a fungal species in more than one azole suggests a multi-drug resistance phenotype that must be further evaluated through antifungal susceptibility testing [82] by the reference microdilution method (EUCAST) [83]. A confirmed resistance phenotype would indicate the presence of azole-resistant fungal species in these settings, thus constituting a higher exposure risk, especially for immunocompromised occupants [57]. One limitation to this characterization is that reference values are defined only for *Aspergillus* sp. and *Candida* sp. [84].

Emergent antifungal resistance in *Aspergillus fumigatus* is the main cause of invasive fungal infections [77,85]. In this study, *Aspergillus* section *Nigri* was identified in one azole media (0.70% on VOR), whereas *Aspergillus* sections *Candidi*, *Fumigati*, *Nidulantes*, *Nigri*, and *Circumdati* were identified in control Sabouraud, in MEA and/or DG18. These results are in line with previous data from dwellings and hospital environment, where no *Aspergillus* species were able to grow on azole-media, despite being observed in Sabouraud, MEA or DG18 [48,54,56,57]. Of note, cryptic *Aspergillus* species might be underestimated in azole-media due to the presence of fast-growing species, such as *Chrysonilia sitophila* and Mucorales group [44,56,57].

Our culture-based methods allowed the identification of the *Aspergillus* section *Fumigati* in a wide number of samples, with molecular tools also detecting this section in different and in a smaller number of samples. Despite this discrepancy, it is of relevance to use both methods, as they provide complimentary information and answer different questions. Indeed molecular tools allow precise, fast, specific and sensitive detection of microorganisms. Importantly, they also can identify dead or dormant microorganisms and can discriminate toxigenic strains from regular strains within some fungal species [86]. Although culture based-methods are selective, revealing only microorganisms able to grow on a particular growth media, therefore, underestimating the total number of microorganisms in samples, these methods are crucial since the viability of bioburden is of critical importance to estimate health risks, as it affects biological mechanisms, such as inflammatory and cytotoxic responses [82,83,87]. This reinforces the idea of combining both molecular and culture-based methods [44].

As previous suggested [88] the sampling approach to assess *SARS-CoV-2* included passive and active sampling methods, swabs being the most common found in the literature [88] and with increased detection when compared with other sampling methods [88,89]. Although the sampling volume from the active sampling was the one recommended [90] and the detection technique was the one widely used for *SARS-CoV-2* detection [88], all the results were negative indicating the efficacy of the present measures in place on the assessed facilities. Further studies, should include a different sampling approach by using glass-fiber and PTFE filters to be employed in low and high-volume air samplers and applying samples pretreatments allowing obtain an increased virus concentrations [91].

5. Conclusions

The present study showed that although the regular cleaning and disinfection procedures effectively removed *SARS-CoV-2* from surfaces, these remained contaminated with other microorganisms besides *SARS-CoV-2*. This can be a result of an increased resistance to biocides, and of the wide range of environmental factors that can contribute to the dissemination of microbial contamination indoors.

Therefore, we recommended that corrective measures should be implemented to reduce bacterial and fungal presence in surfaces to avoid contamination in the air due to resuspension. Additional studies aiming at correlating air and surfaces microorganisms' burden can be a valuable tool in finding the contamination sources.

Author Contributions: Conceptualization, C.V.; methodology, C.V.; formal analysis, C.V., R.P., M.D., B.G., L.A.C., M.B., E.C. and A.Q.G.; investigation, C.V.; resources, C.V.; writing—original draft preparation, C.V., R.P., M.D. and L.A.C.; writing—review and editing, C.V., M.B. and A.Q.G.; supervision, C.V.; project administration, C.V.; funding acquisition, C.V. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Instituto Politécnico de Lisboa, Lisbon, Portugal for funding the Project "IPL MOMENTO ZERO" (ESTeSL/IPL/2020). H&TRC authors gratefully acknowledge the FCT/MCTES national support through the UIDB/05608/2020 and UIDP/05608/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. I have full control of all primary data and permission is given to the journal to review the data if requested.

References

1. Zhang, S.; Zheng, J.; Wu, Y. Field study of air environment perceptions and influencing factors in waiting spaces of general hospitals in winter cities. *Build. Environ.* **2020**, *183*, 107203. [CrossRef]
2. Śmiełowska, M.; Marć, M.; Zabiegała, B. Indoor air quality in public utility environments—A review. *Environ. Sci. Pollut. Res.* **2017**, *24*, 11166–11176. [CrossRef]
3. Yin, H.; Liu, C.; Zhang, L.; Li, A.; Ma, Z. Measurement and evaluation of indoor air quality in naturally ventilated residential buildings. *Indoor Built Environ.* **2019**, *28*, 1307–1323. [CrossRef]
4. Hwang, S.H.; Roh, J.; Park, W.M. Evaluation of PM10, CO2, airborne bacteria, TVOCs, and formaldehyde in facilities for susceptible populations in South Korea. *Environ. Pollut.* **2018**, *242*, 700–708. [CrossRef]
5. Brągoszewska, E.; Biedroń, I.; Kozielska, B.; Pastuszka, J.S. Microbiological indoor air quality in an office building in Gliwice, Poland: Analysis of the case study. *Air Qual. Atmos. Health* **2018**, *11*, 729–740. [CrossRef]
6. Guo, H.; Lee, S.C.; Chan, L.Y. Indoor air quality investigation at air-conditioned and non-air-conditioned markets in Hong Kong. *Sci. Total Environ.* **2004**, *323*, 87–98. [CrossRef]
7. Majd, E.; McCormack, M.; Davis, M.; Curriero, F.; Berman, J.; Connolly, F.; Leaf, P.; Rule, A.; Green, T.; Clemons-Erby, D.; et al. Indoor air quality in inner-city schools and its associations with building characteristics and environmental factors. *Environ. Res.* **2019**, *170*, 83–91. [CrossRef]
8. Van Tran, V.; Park, D.; Lee, Y.C. Indoor Air Pollution, Related Human Diseases, and Recent Trends in the Control and Improvement of Indoor Air Quality. *Int. J. Environ. Res. Public Health* **2020**, *17*, 2927. [CrossRef]
9. Cincinelli, A.; Martellini, T. Indoor air quality and health. *Int. J. Environ. Res. Public Health* **2017**, *14*, 1286. [CrossRef] [PubMed]
10. USEPA. Introduction to Indoor Air Quality. Available online: https://www.epa.gov/indoor-air-quality-iaq/introduction-indoor-air-quality (accessed on 21 January 2021).
11. Matos, J.; Brantes, J.; Cunha, A.M. *Qualidade do Ar em Espaços Interiores Um Guia Técnico 2010*; Agência Port. do Ambient: Lisbon, Portugal, 2010.
12. Duarte, N.; Roa, S.; Francy, M.-C. Microbiological Air Quality in an Optical Clinic. *Ciencia y Tecnología para la Salud Visual y Ocular* **2019**, *17*, 19–28. [CrossRef]
13. Douwes, J.; Thorne, P.; Pearce, N.; Heederik, D. Bioaerosol health effects and exposure assessment: Progress and prospects. *Ann. Occup. Hyg.* **2003**, *47*, 187–200. [CrossRef] [PubMed]
14. Moelling, K.; Broecker, F. Air Microbiome and Pollution: Composition and Potential Effects on Human Health, including SARS Coronavirus Infection. *J. Environ. Public Health* **2020**, *2020*, 1646943. [CrossRef]
15. Ijaz, M.K.; Zargar, B.; Wright, K.E.; Rubino, J.R.; Sattar, S.A. Generic aspects of the airborne spread of human pathogens indoors and emerging air decontamination technologies. *Am. J. Infect. Control* **2016**, *44*, S109–S120. [CrossRef]
16. Walsh, T.J.; Anaissie, E.J.; Denning, D.W.; Herbrecht, R.; Kontoyiannis, D.P.; Marr, K.A.; Morrison, V.A.; Segal, B.H.; Steinbach, W.J.; Stevens, D.A.; et al. Treatment of aspergillosis: Clinical practice guidelines of the infectious diseases society of America. *Clin. Infect. Dis.* **2008**, *46*, 327–360. [CrossRef]
17. Sabino, R. Exposure to Fungi in Health Care Facilities. *Encycl. Mycol.* **2021**, *2*, 1–10. [CrossRef]
18. WHO. Antimicrobial Resistance. Available online: https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance (accessed on 5 February 2021).
19. Stop neglecting fungi. *Nat. Microbiol.* **2017**, *2*, 17120. [CrossRef] [PubMed]
20. Azuma, K.; Yanagi, U.; Kagi, N.; Kim, H.; Ogata, M.; Hayashi, M. Environmental factors involved in SARS-CoV-2 transmission: Effect and role of indoor environmental quality in the strategy for COVID-19 infection control. *Environ. Health Prev. Med.* **2020**, *25*, 1–17. [CrossRef]
21. Di Giulio, M.; Grande, R.; Di Campli, E.; Di Bartolomeo, S.; Cellini, L. Indoor air quality in university environments. *Environ. Monit. Assess.* **2010**, *170*, 509–517. [CrossRef] [PubMed]

22. Erlandson, G.; Magzamen, S.; Carter, E.; Sharp, J.L.; Reynolds, S.J.; Schaeffer, J.W. Characterization of indoor air quality on a college campus: A pilot study. *Int. J. Environ. Res. Public Health* **2019**, *16*, 2721. [CrossRef] [PubMed]
23. Haleem, A.M.; Hassan, D.M.A.; Al-hiyaly, S.A.K. Comparative Assessment of Microbial Contamination from Swabs collected within University Facilities. *J. Health Sci.* **2013**, *3*, 25–28. [CrossRef]
24. Ross, A.A.; Neufeld, J.D. Microbial biogeography of a university campus. *Microbiome* **2015**, *3*, 66. [CrossRef]
25. Lou, X.; Fang, Z.; Gong, C. Assessment of culturable airborne fungi in a university campus in Hangzhou, southeast China. *Afr. J. Microbiol. Res.* **2012**, *6*, 1197–1205. [CrossRef]
26. McEwen, S.A.; Collignon, P.J. Antimicrobial Resistance: A One Health Perspective. *Antimicrob. Resist. Bact. Livest. Companion Anim.* **2018**, *6*, 521–547. [CrossRef]
27. MStryjakowska-Sekulska, A.; Piotraszewska-Pająk, A.; Szyszka, M.N.; Filipiak, M. Microbiological quality of indoor air in University Rooms. *Pol. J. Environ.* **2007**, *16*, 623–632.
28. Onet, A.; Ilies, D.C.; Buhas, S.; Rahota, D.; Ilies, A.; Baias, S.; Marcu, F.; Herman, G.V. Microbial air contamination in indoor environment of university sports hall. *J. Environ. Prot. Ecol.* **2018**, *19*, 694–703.
29. Zulfakar, S.S.; Abu Hassan, M.F.; Abu Bakar, N.F. Microbiological Assessment of Selected Laboratories at a Local University in Malaysia. *J. Sains Kesihat. Malays.* **2019**, *17*, 119–126. [CrossRef]
30. Kic, P.; Růžek, L. The microbiological environment in specific rooms of a university campus. *Agron. Res.* **2014**, *12*, 837–842.
31. Hayleeyesus, S.F.; Manaye, A.M. Microbiological quality of indoor air in University libraries. *Asian Pac. J. Trop. Biomed.* **2014**, *4*, S312–S317. [CrossRef] [PubMed]
32. Asif, A.; Zeeshan, M.; Jahanzaib, M. Assessment of indoor and outdoor microbial air quality of cafeterias of an educational institute. *Atmos. Pollut. Res.* **2019**, *10*, 531–536. [CrossRef]
33. Jurado, S.R.; Bankoff, A.D.P.; Sanchez, A. Indoor air quality in Brazilian universities. *Int. J. Environ. Res. Public Health* **2014**, *11*, 7081–7093. [CrossRef]
34. Amira Idris, S.; Hanafiah, M.M.; Ismail, M.; Abdullah, S.; Khan, M.F. Laboratory air quality and microbiological contamination in a university building. *Arab. J. Geosci.* **2020**, *13*, 580. [CrossRef]
35. Li, Y.; Ge, Y.; Wu, C.; Guan, D.; Liu, J.; Wang, F. Assessment of culturable airborne bacteria of indoor environments in classrooms, dormitories and dining hall at university: A case study in China. *Aerobiologia* **2020**, *36*, 313–324. [CrossRef]
36. Crowe, J.; Schnaubelt, A.T.; Scott, S.-B.; Kathleen, A.; Bai, J.; Eske, T.; Molly, N.; Catherine, P.; White, B.; Crotts-Hannibal, B.; et al. Pilot Program for Test-Based SARS-CoV-2 Screening and Environmental Monitoring in an Urban Public School District. *medRxiv* **2021**. [CrossRef]
37. Viegas, C.; Dias, M.; Gomes, B.; Pimenta, R.; Oliveira, K.; Delgadinho, M.; Ginete, C.; Abreu, R.; Almeida-Silva, M.; Almeida, A.; et al. Towards and integrated approach on Occupational Health to tackle COVID19 pandemic. 2021, submitted for publication.
38. United States Environmental Protection Agency. *EPA Observational Economy Series Volume 1: Composite Sampling*; EPA: Washington, DC, USA, 1995; Volume 1.
39. EUCAST (European Committee on Antimicrobial Susceptibility Testing). Available online: https://www.eucast.org (accessed on 21 January 2021).
40. De Hoog, C.; Guarro, J.; Gene, G.; Figueras, M. *Atlas of Clinical Fungi—The Ultimate Benchtool for Diagnosis*, version 4.1.4; Centraalbureau voor Schimmelcultures: Utrecht, The Netherlands, 2016.
41. Viegas, C.; Sousa, P.; Dias, M.; Caetano, L.A.; Ribeiro, E.; Carolino, E.; Twarużek, M.; Kosicki, R.; Viegas, S. Bioburden contamination and Staphylococcus aureus colonization associated with firefighter's ambulances. *Environ. Res.* **2021**, *197*, 111125. [CrossRef] [PubMed]
42. Viegas, C.; Coggins, A.M.; Faria, T.; Caetano, L.A.; Gomes, A.Q.; Sabino, R.; Verissimo, C.; Roberts, N.; Watterson, D.; MacGilchrist, C.; et al. Fungal burden exposure assessment in podiatry clinics from Ireland. *Int. J. Environ. Health Res.* **2018**, *28*, 167–177. [CrossRef] [PubMed]
43. World Health Organisation. *WHO Guidelines for Indoor Air Quality: Dampness and Mould*; WHO Regional Office for Europe: Copenhagen, Denmark, 2009; ISBN 978 92 890 4168 3.
44. Viegas, C.; Caetano, L.A.; Viegas, S. Occupational exposure to Aspergillus section Fumigati: Tackling the knowledge gap in Portugal. *Environ. Res.* **2021**, *194*, 110674. [CrossRef] [PubMed]
45. Viegas, C. *Sampling Methods for an Accurate Mycobiota Occupational Exposure Assessment—Overview of Several Ongoing Projects*; CRC Press: London, UK, 2018; ISBN 9781138542037.
46. Viegas, C.; Almeida, B.; Monteiro, A.; Paciência, I.; Rufo, J.; Aguiar, L.; Lage, B.; Diogo Gonçalves, L.M.; Caetano, L.A.; Carolino, E.; et al. Exposure assessment in one central hospital: A multi-approach protocol to achieve an accurate risk characterization. *Environ. Res.* **2020**, *181*, 108947. [CrossRef]
47. Viegas, C.; Dias, M.; Carolino, E.; Sabino, R. Culture media and sampling collection method for aspergillus spp. Assessment: Tackling the gap between recommendations and the scientific evidence. *Atmosphere* **2021**, *12*, 23. [CrossRef]
48. Viegas, C.; Almeida, B.; Monteiro, A.; Paciência, I.; Rufo, J.C.; Carolino, E.; Quintal-Gomes, A.; Twarużek, M.; Kosicki, R.; Marchand, G.; et al. Settled dust assessment in clinical environment: Useful for the evaluation of a wider bioburden spectrum. *Int. J. Environ. Health Res.* **2021**, *31*, 160–178. [CrossRef]

49. Viegas, C.; Ramalho, I.; Alves, M.; Faria, T.; Caetano, L.A.; Viegas, S. Electrostatic dust cloth: A new sampling method for occupational exposure to bioaerosols. Review of literature. In Proceedings of the SHO-2017—International Symposium on Occupational Safety and Hygiene, Guimarães, Portugal, 10–11 April 2017; pp. 39–41.
50. Lednicky, J.A.; Lauzard, M.; Fan, Z.H.; Jutla, A.; Tilly, T.B.; Gangwar, M.; Usmani, M.; Shankar, S.N.; Mohamed, K.; Eiguren-Fernandez, A.; et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *Int. J. Infect. Dis.* **2020**, *100*, 476–482. [CrossRef] [PubMed]
51. Monteiro, A.; Almeida, B.; Paciência, I.; Rufo, J.C.; Ribeiro, E.; Carolino, E.; Viegas, C.; Uva, A.S.; Verde, S.C. Bacterial contamination in health care centers: Differences between urban and rural settings. *Atmosphere* **2021**, *12*, 450. [CrossRef]
52. Viegas, C.; Almeida-Silva, M.; Gomes, A.Q.; Wolterbeek, H.T.; Almeida, S.M. Fungal contamination assessment in portuguese elderly care centers. *J. Toxicol. Environ. Health Part A Curr. Issues* **2014**, *77*, 14–23. [CrossRef]
53. Pinheiro, A.C.; Viegas, C.; Viegas, S.; Veríssimo, C.; Brandão, J.; MacEdo, M.F. Indoor air quality in portuguese archives: A snapshot on exposure levels. *J. Toxicol. Environ. Health Part A Curr. Issues* **2012**, *75*, 1359–1370. [CrossRef]
54. Viegas, C.; Dias, M.; Monteiro, A.; Faria, T.; Lage, J.; Carolino, E.; Caetano, L.A.; Gomes, A.Q.; Almeida, S.M.; Verde, S.C.; et al. Bioburden in sleeping environments from Portuguese dwellings. *Environ. Pollut.* **2021**, *273*, 116417. [CrossRef]
55. Canha, N.; Alves, A.C.; Marta, C.S.; Lage, J.; Belo, J.; Faria, T.; Cabo Verde, S.; Viegas, C.; Alves, C.; Almeida, S.M. Compliance of indoor air quality during sleep with legislation and guidelines—A case study of Lisbon dwellings. *Environ. Pollut.* **2020**, *264*, 114619. [CrossRef] [PubMed]
56. Viegas, C.; Twaruzek, M.; Lourenço, R.; Dias, M.; Almeida, B.; Caetano, L.A.; Carolino, E.; Gomes, A.Q.; Kosicki, R.; Soszczyńska, E.; et al. Bioburden assessment by passive methods on a clinical pathology service in one central hospital from lisbon: What can it tell us regarding patients and staff exposure? *Atmosphere* **2020**, *11*, 351. [CrossRef]
57. Viegas, C.; Almeida, B.; Monteiro, A.; Caetano, L.A.; Carolino, E.; Gomes, A.Q.; Twarużek, M.; Kosicki, R.; Marchand, G.; Viegas, S. Bioburden in health care centers: Is the compliance with Portuguese legislation enough to prevent and control infection? *Build. Environ.* **2019**, *160*, 106226. [CrossRef]
58. Viegas, C.; Faria, T.; Pacífico, C.; Dos Santos, M.; Monteiro, A.; Lança, C.; Carolino, E.; Viegas, S.; Cabo Verde, S. Microbiota and Particulate Matter Assessment in Portuguese Optical Shops Providing Contact Lens Services. *Healthcare* **2017**, *5*, 24. [CrossRef]
59. Ramos, C.A.; Viegas, C.; Verde, S.C.; Wolterbeek, H.T.; Almeida, S.M. Characterizing the fungal and bacterial microflora and concentrations in fitness centres. *Indoor Built Environ.* **2016**, *25*, 872–882. [CrossRef]
60. Almeida-Silva, M.; Almeida, S.M.; Pegas, P.N.; Nunes, T.; Alves, C.A.; Wolterbeek, H.T. Exposure and dose assessment to particle components among an elderly population. *Atmos. Environ.* **2015**, *102*, 156–166. [CrossRef]
61. Cabo Verde, S.; Almeida, S.M.; Matos, J.; Guerreiro, D.; Meneses, M.; Faria, T.; Botelho, D.; Santos, M.; Viegas, C. Microbiological assessment of indoor air quality at different hospital sites. *Res. Microbiol.* **2015**, *166*, 557–563. [CrossRef]
62. Wolny-Koładka, K.; Malinowski, M.; Pieklik, A.; Kurpaska, S. Microbiological air contamination in university premises and the evaluation of drug resistance of staphylococci occurring in the form of a bioaerosol. *Indoor Built Environ.* **2019**, *28*, 235–246. [CrossRef]
63. Grisoli, P.; Rodolfi, M.; Chiara, T.; Zonta, L.A.; Dacarro, C. Evaluation of microbiological air quality and of microclimate in university classrooms. *Environ. Monit. Assess.* **2012**, *184*, 4171–4180. [CrossRef] [PubMed]
64. Viegas, C.; Faria, T.; Meneses, M.; Carolino, E.; Viegas, S.; Gomes, A.Q.; Sabino, R. Analysis of surfaces for characterization of fungal burden—Does it matter? *Int. J. Occup. Med. Environ. Health* **2016**, *29*, 623–632. [CrossRef] [PubMed]
65. Srikanth, P.; Sudharsanam, S.; Steinberg, R. Bio-Aerosols in Indoor Environment: Composition, Health Effects and Analysis. *Indian J. Med. Microbiol.* **2008**, *26*, 302–312. [CrossRef]
66. Kim, K.H.; Kabir, E.; Jahan, S.A. Airborne bioaerosols and their impact on human health. *J. Environ. Sci.* **2018**, *67*, 23–35. [CrossRef] [PubMed]
67. Nazaroff, W.W. Indoor bioaerosol dynamics. *Indoor Air* **2016**, *26*, 61–78. [CrossRef] [PubMed]
68. Moldoveanu, A.M. Biological Contamination of Air in Indoor Spaces. In *Current Air Quality Issues*; Farhad, N., Ed.; INTECHoPE: London, UK, 2015; ISBN 978-953-51-2180-0.
69. Liofilchem Dichloran Glycerol (DG18) Agar Base. Available online: http://www.liofilchem.net/login/pd/ts/620238_TS.pdf (accessed on 16 April 2021).
70. Viegas, C.; Alves, C.; Carolino, E.; Pinheiro, C.; Rosado, L.; Silva-Santos, C. Assessment of fungal contamination in a group of Lisbon's gymnasiums with a swimming pool. *Ital. J. Occup. Environ. Hyg.* **2011**, *2*, 15–20.
71. Viegas, C.; Faria, T.; Monteiro, A.; Caetano, L.A.; Carolino, E.; Gomes, A.Q.; Viegas, S. A novel multi-approach protocol for the characterization of occupational exposure to organic dust-Swine production case study. *Toxics* **2018**, *6*, 5. [CrossRef]
72. Reboux, G.; Bellanger, A.P.; Roussel, S.; Grenouillet, F.; Sornin, S.; Piarroux, R.; Dalphin, J.C.; Millon, L. Indoor mold concentration in Eastern France. *Indoor Air* **2009**, *19*, 446–453. [CrossRef]
73. Adams, R.I.; Miletto, M.; Taylor, J.W.; Bruns, T.D. The diversity and distribution of fungi on residential surfaces. *PLoS ONE* **2013**, *8*, e78866. [CrossRef] [PubMed]
74. Nevalainen, A.; Täubel, M.; Hyvärinen, A. Indoor fungi: Companions and contaminants. *Indoor Air* **2015**, *25*, 125–156. [CrossRef] [PubMed]
75. WHO. *Development of WHO Guidelines for Indoor Air Quality—Report on a Working Group Meeting*; World Health Organization Regional Office for Europe: Copenhagen, Denmark, 2006; pp. 1–27.

76. Varga, J.; Baranyi, N.; Chandrasekaran, M.; Vágvölgyi, C.; Kocsubé, S. Mycotoxin producers in the Aspergillus genus: An update. *Acta Biol. Szeged.* **2015**, *59*, 151–167.
77. Sabino, R.; Veríssimo, C.; Viegas, C.; Viegas, S.; Brandão, J.; Alves-Correia, M.; Borrego, L.M.; Clemons, K.V.; Stevens, D.A.; Richardson, M. The role of occupational Aspergillus exposure in the development of diseases. *Med. Mycol.* **2019**, *57*, S196–S205. [CrossRef] [PubMed]
78. Viegas, C.; Faria, T.; Caetano, L.A.; Carolino, E.; Gomes, A.Q.; Viegas, S. Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings? *J. Occup. Environ. Hyg.* **2017**, *14*, 771–785. [CrossRef] [PubMed]
79. Viegas, C.; Santos, P.; Almeida, B.; Monteiro, A.; Carolino, E.; Gomes, A.Q.; Viegas, S. Electrostatic dust collector: A passive screening method to assess occupational exposure to organic dust in primary health care centers. *Air Qual. Atmos. Health* **2019**, *12*, 573–583. [CrossRef]
80. Perlin, D.S.; Rautemaa-Richardson, R.; Alastruey-Izquierdo, A. The global problem of antifungal resistance: Prevalence, mechanisms, and management. *Lancet Infect. Dis.* **2017**, *17*, e383–e392. [CrossRef]
81. Fisher, M.C.; Hawkins, N.J.; Sanglard, D.; Gurr, S.J. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science* **2018**, *360*, 739–742. [CrossRef]
82. Viegas, C.; Twarużek, M.; Dias, M.; Almeida, B.; Carolino, E.; Kosicki, R.; Soszczyńska, E.; Grajewski, J.; Caetano, L.A.; Viegas, S. Assessment of the microbial contamination of mechanical protection gloves used on waste sorting industry: A contribution for the risk characterization. *Environ. Res.* **2020**, *189*, 109881. [CrossRef]
83. Viegas, C.; Dias, M.; Almeida, B.; Aranha Caetano, L.; Carolino, E.; Quintal Gomes, A.; Twarużek, M.; Kosicki, R.; Grajewski, J.; Marchand, G.; et al. Are workers from waste sorting industry really protected by wearing Filtering Respiratory Protective Devices? The gap between the myth and reality. *Waste Manag.* **2020**, *102*, 856–867. [CrossRef]
84. EUCAST. Overview of Antifungal ECOFFs and Clinical Breakpoints for Yeasts, Moulds and Dermatophytes Using the EUCAST E.Def 7.3, E.Def 9.3 and E.Def 11.0 Procedures. Version 2. Available online: https://www.eucast.org (accessed on 21 January 2021).
85. CDC. Antifungal Resistance. Available online: https://www.cdc.gov/fungal/antifungal-resistance.html (accessed on 20 June 2021).
86. MacNeil, L.; Kauri, T.; Robertson, W. Molecular techniques and their potential application in monitoring the microbiological quality of indoor air. *Can. J. Microbiol.* **1995**, *41*, 657–665. [CrossRef] [PubMed]
87. Madsen, A.M.; Frederiksen, M.W.; Jacobsen, M.H.; Tendal, K. Towards a risk evaluation of workers' exposure to handborne and airborne microbial species as exemplified with waste collection workers. *Environ. Res.* **2020**, *183*, 109177. [CrossRef] [PubMed]
88. Pena, P.; Morais, J.; Quintal Gomes, A.; Viegas, C. Sampling methods and assays applied in *SARS-CoV-2* exposure assessment. *Sci. Total Environ.* **2021**, *775*, 145903. [CrossRef]
89. Moreno, T.; Pintó, R.M.; Bosch, A.; Moreno, N.; Alastuey, A.; Minguillón, M.C.; Anfruns-Estrada, E.; Guix, S.; Fuentes, C.; Buonanno, G.; et al. Tracing surface and airborne SARS-CoV-2 RNA inside public buses and subway trains. *Environ. Int.* **2021**, *147*, 106326. [CrossRef] [PubMed]
90. Bertin Instruments. Air Monitoring—Coriolis Air Samplers Collect Biological Particles in the Air Which Offer New Perspectives for the Control of Airborne Contamination Thanks to Its Liquid Sample. 2020. Available online: https://www.bertininstruments.com/products-range/air-samplers/ (accessed on 21 January 2021).
91. Robotto, A.; Civra, A.; Quaglino, P.; Polato, D.; Brizio, E.; Lembo, D. SARS-CoV-2 airborne transmission: A validated sampling and analytical method. *Environ. Res.* **2021**, *200*, 111783. [CrossRef] [PubMed]