INSTITUTO POLITÉCNICO DE LISBOA

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA

MICROBIOLOGICAL CONTAMINATION ASSESSMENT IN HIGHER EDUCATION INSTITUTES

RAQUEL FILIPA LOURENÇO PIMENTA

SUPERVISOR: PhD. CARLA VIEGAS – ESTeSL - IPL

MASTER'S DEGREE IN CLINICAL LABORATORY TECHNOLOGIES

Lisboa, 2021

INSTITUTO POLITÉCNICO DE LISBOA

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA

MICROBIOLOGICAL CONTAMINATION ASSESSMENT IN HIGHER EDUCATION INSTITUTES

RAQUEL FILIPA LOURENÇO PIMENTA

SUPERVISOR: PhD. CARLA VIEGAS – ESTeSL - IPL

MASTER'S DEGREE IN CLINICAL LABORATORY TECHNOLOGIES

(this version include the criticism and suggestions made by the jury)

Lisboa, 2021

MICROBIOLOGICAL CONTAMINATION ASSESSMENT IN HIGHER EDUCATION INSTITUTES

This work was financially supported by Instituto Politécnico de Lisboa (IPL) for the Project “IPL MOMENTO ZERO” (ESTeSL/IPL/BI/2020). In the scope of Health and Technology Research Center (H&TRC) research projects the author gratefully acknowledges the Fundação para a Ciência e Tecnologia – Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES) national support through the UIDB/05608/2020 and UIDP/05608/2020. Scientific support and guidance was given by CEDOC, NOVA Medical School, NMS, Universidade Nova de Lisboa, as well as Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa.

Acknowledgements

I want to thank a few people for supporting me during these two years, allowing the end of this journey to become a reality.

First of all, I want to thank to Professor Carla Viegas for your availability, patience, and interest in clarifying doubts and for your constructive comments in the development of the dissertation. I am also grateful for your contagious curiosity for the investigation and the fact that you believe in me and encourage me to do a good job. Thank you for introducing me to other projects that contributed to my professional and personal development. It was a pleasure working with you.

To thank Marta Dias who received me in the laboratory and helped me with all the laboratory work, being always available to answer questions. It was great working with you.

To Bianca Gomes and Ana Simões for your support with laboratory tasks. It was a pleasure working with you.

I also thank to Escola Superior de Tecnologia da Saúde de Lisboa and Instituto Politécnico de Lisboa, in particular the H&TRC research group and all its workers, for allowing this work to be carried out.

To Professor Elisabete Carolino for your help with statistical results.

I could not fail to thank to my family, especially my parents, who encouraged me and allowed me to continue studying for all these years. Without your unconditional love, support, patience, and advice none of this would have been possible.

To my brother Ricardo, for being positive when I face challenges.

Last but not least, I want to thank my grandmother Irene for all your concern, help and love.

To all of you, thank you so much.

Abstract

In higher education institutes (HEI), microbiological contamination can impair indoor air quality (IAQ), affecting the health, performance and productivity of the many occupants who spend a lot of time inside the building.

The aim of this study was to assess fungi, bacteria, and the *SARS-COV-2* virus in ten units of HEI. Azole resistance screening was also analyzed.

Fungal and bacterial contamination on surfaces was characterized by culture-based methods. Air and surface samples were also used for the detection of *SARS-CoV-2* and for the identification of *Aspergillus* section *Fumigati* by molecular tools.

On surfaces, total bacterial contamination ranged from 1 x 10^3 to 3.1 x 10^6 CFU.m^{-2}, while Gram-negative bacteria ranged between 0 to 1.9 x 10^4 CFU.m^{-2}. The total fungal contamination on the surfaces ranged from 0 to 1.5 x 10^5 CFU.m^{-2} on malt extract agar (MEA) culture media, and from 0 to 1.8 × 10^5 CFU.m^{-2} on Dichloran glycerol agar (DG18) culture media. *Cladosporium* sp. was the most prevalent fungal species found. *Aspergillus* sections were identified on MEA and DG18, being only section *Nigri* observed in one supplemented media used for the azole screening. *Aspergillus* section *Fumigati* was not detected on air samples, whereas it was on two surface samples. The presence of *SARS-CoV-2* was not detected.

In conclusion, although cleaning and disinfection procedures are done regularly due to the COVID-19 pandemic, being effective in eliminating *SARS-CoV-2*, the surfaces were often contaminated with fungi and bacteria.

Keywords

Higher education institutes; Fungi; Bacteria; *SARS-CoV-2;* Azole fungal resistance.

Resumo

Em instituições de ensino superior (IES), a contaminação microbiológica pode prejudicar a qualidade do ar interior (QAI), afetando a saúde, desempenho e produtividade dos muitos ocupantes que passam muito tempo dentro do edifício.

O objetivo deste estudo foi avaliar fungos, bactérias e do vírus *SARS-COV-2* em dez unidades de IES. A resistência aos azóis também foi analisada.

A contaminação fúngica e bacteriana nas superfícies foi caracterizada por métodos baseados em cultura. Amostras de ar e de superfície foram também usadas para a deteção de *SARS-CoV-2* e para a identificação de *Aspergillus* section *Fumigati* por ferramentas moleculares.

Nas superfícies, a contaminação total bacteriana variou de 1 x 10^3 a 3.1 x 10^6 UFC.m^{-2}, enquanto as bactérias Gram-negativas variaram entre 0 a 1.9 x 10^4 UFC.m^{-2}. A contaminação fúngica total nas superfícies variou de 0 a 1.5 x 10^5 UFC.m^{-2} no meio de cultura ágar extrato de malte (MEA) e de 0 to 1.8 × 10^5 UFC.m^{-2} no meio de cultura agar diclorano-glicerol (DG18). *Cladosporium* sp. foi a espécie fúngica encontrada em maior prevalência. As espécies de *Aspergillus* foram identificadas em MEA e DG18, sendo apenas observado o complexo *Nigri* num meio suplementado usado para a resistência aos azóis. *Aspergilllus* section *Fumigati* não foi detectado nas amostras de ar, enquanto em duas amostras de superfície foi encontrado. A presença de *SARS-CoV-2* não foi detetada.

Concluindo, embora os procedimentos de limpeza e desinfeção sejam feitos regularmente, sendo eficazes na eliminação de *SARS-CoV-2*, as superfícies apresentaram frequente contaminação fúngica e bacteriana.

Palavras-chave

Institutos de ensino superior; Fungos; Bactérias; *SARS-CoV-2;* Resistência fúngica aos azóis.

Index

Tables Index

Figures Index

Acronyms List

aW - Water activity
CFU – Colony-forming units
DG18 – Dichloran glycerol agar
HEI – Higher education institute
IAP - Indoor air pollution
IAQ- Indoor air quality
ITR – Itraconazole
MEA – Malt extract agar
PB – Presidency building
PCR - Polymerase chain reaction
POS – Posaconazole
qPCR – Real-time quantitative PCR
RT-qPCR – Reverse transcription quantitative real-time PCR
SAB – Sabouraud dextrose agar
SS – Social Services
TSA – Tryptic soy agar
VOR – Voriconazole
VRBA – Violet red bile agar
WHO - World Health Organization

1. Introduction

People spend most of their time in indoor environments, such as offices, schools, and homes, so the indoor air quality (IAQ) is one of the main factors affecting health, wellbeing and productivity (1–4). In recent years, there has been a growing interest in microbiological air quality studies (2,4).

Higher education institutes (HEI) comprise a high population density that spent a lot of time inside the building (5–7).

Health effects may be experienced soon after exposure (immediately) or, possibly, years later (long-term) (8). Exposure to microorganisms 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 (mycotoxins) (9–11). 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 (9,12–14). Another major concern is the emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance, which makes it difficult or impossible to treat common infections (15). Furthermore, in the last year, the emergence of *SARS-CoV-2* virus has caused of several damages in the world (including many deaths), and its detection in different environments must be studied to guarantee public health (16). Thus, this effects can increase absenteeism and reduce academic performance (7,17,18).

Although microorganisms are ubiquitous, an increase of their concentration could represent a risk factor (18). In indoor air they are mainly influenced by human occupancy and their activities, building materials, furnishings, ventilation and outdoor air (18,19). Thus, microorganisms can be released into the air forming bioaerosols from almost surfaces (20). Besides that, microbial particles are constantly removed for the air and deposited on surfaces (20). Therefore, microbiological contamination on surfaces is also an important tool to identify sources of contamination and evaluate the effectiveness of cleaning and disinfection procedures on surfaces (21).

For these reasons, the assessment of microbial contamination is crucial to providing a safe environment.

1.1. Objectives

The aim of the study is to assess the presence of fungi, bacteria, and the *SARS-COV-2* virus in HEI. To achieve the main objective, it is necessary to accomplish other specific objectives, such as collecting air and surface samples to carry out the characterization of the fungi and bacteria and the detection of *SARS-CoV-2*; performing the morphological identification of fungi, giving special emphasis to *Aspergillus* sections; and screening the azole resistance.

2. State of the Art

This chapter presents the state of the art regarding the subject under study, addressing some themes such as the importance of IAQ and the effect of microbiological contaminants.

2.1. Indoor Air Quality

Problems related to poor IAQ have existed ever since human beings started building shelters to protect themselves from the harshness of the natural environment (22). During history, the man has known that polluted air may cause adverse effects on health (23). In the last few decades, several studies were conducted to understand the impact of IAQ in public health in different environments, including residential building, shopping malls, schools, health care centers, offices, museums, libraries, temples, and churches, etc. (24–30). Indoor environments conditions contribute greatly to comfort, health and wellbeing of building occupants (3,10,31). However, the indoor air pollution (IAP), that refers to the existence of pollutants, can influence negatively the IAQ and are harmful to the human body (3,10). Most people spend around 90% of their time indoors, mainly at home or in the workplace (10). Because of this, IAQ has emerged and received increasing attention (3,10,31).

One of the most important parameters influencing IAQ is indoor air pollutants, which are divided into chemical and biological (3,10). Biological pollutants that have health relevance exhibit enormous heterogeneity, including biological allergens (animal dander and cat saliva, house dust, cockroaches, mites, and pollen) and microorganisms (viruses, fungi, and bacteria) (10,32–34).

2.2. Microbiological Contaminants

Microorganisms are ubiquitous (20,34,35). Their sources varied with the type of microorganisms and can be carried by people, animals and soil and plants (10).

Wherever microbial sources are present, these particles can be released into the air forming microbiological aerosols (20). They can be both actively (e.g. by breathing, coughing) and passively (through meteorological processes) emitted to the atmosphere from almost all surfaces (20). Independent of the air turbulence, microbial particles are constantly removed from the air due to gravitational sedimentation, interception, impaction or precipitation processes, ending in both cases in deposition on surfaces as ‘free’ microbial particles or inside water droplets or ice crystals (20). Therefore, microbial survival decreases when the air is deprived of nutrient sources, with

constantly changing moisture content, and a wide range of different stress factors (20). However, microbial particles are capable of preserving their viability and, with it, also all related biological properties (including infectivity, toxicity, allergenicity, etc.) (20). If deposited on surfaces, the microbial particles may also maintain their viability for a long period of time and, when resuspended in the air, may still pose a serious threat to the exposed individuals (20). Thus, the fact that inhalation of immunologically active particles can be responsible for numerous adverse health conditions, the relationship between their stability on surfaces and viability in the air is very important from the exposure assessment point of view (20). Furthermore, surfaces analysis complements microbiological characterization of the air and is used in order to identify contamination sources, and to evaluate efficacy of surface cleaning and disinfection procedures (21).
The three main types of microbiological contaminants are bacteria, fungi and viruses and are described below (35).

2.2.1. Fungi

Fungi are present in all buildings, comprising an abundance of species (34,36). The most important source of indoor fungi is outdoor air and are present in lower concentrations indoors than outdoors (36,37). They may be transported into buildings on the surface of new materials or by humans (clothes, skin, hair and shoes) or by pets (34,36). They may also penetrate buildings through ventilation (34). They are found in the dust and surfaces of every house, including those with no problems with damp (34). However, sometimes the indoor fungal levels are higher than the outdoor levels, and this could indicate an indoor fungal source (37).
Once fungi are indoors, fungal growth can be promoted in the presence of moisture, and many fungi grow readily on any surface that becomes wet or moistened (34,36). The minimum requirements for temperature, pH, light, and availability of nutrients are usually present in building (34,36). However, although temperature, nutrients, and water activity are not crucial, they may affect the rate of growth (34,36). Furthermore, factors contributing to the fungal occurrence indoors and to their stage include ‘major’ phenomena, such as climate, geography, season, and location, and ‘minor’ factors which refer to individual buildings and their occupants, such as building construction and maintenance, current and historical use of the building, ventilation, moisture control, surface materials, occupants themselves, and their activities and lifestyles (36).
The most common adverse health effects associated with fungi in indoor environments are various respiratory conditions (e.g. worsening asthma symptoms and lung function), followed by inflammatory, allergic and toxin effects (36,38,39). Furthermore,

some indoor fungi, when ingested or inhaled, could produce mycotoxins defined as toxic secondary biomolecules produced by fungus or molds that interfere with RNA synthesis and can cause DNA damage (40–42). The health effects of mycotoxins are diverse enough to include weakened immune systems, allergies or irritations, many identifiable diseases, and even death (41). Besides that, the rapid emergence of drug resistant pathogenic fungi severely limits therapy (43–45). Multidrug resistance can eliminate treatment option entirely, which has a devastating effect on patient outcome (43–45). Over 300 million people suffer from serious fungal-related diseases, and fungi collectively kill over 1.6 million people annually (45). Fungi not only have adverse effects on health but also create damage to building and to their materials (34,35).

The genus *Aspergillus* is known to be one of the most frequently airborne fungi with pathogenicity found in indoor environments worldwide and for that reason will be discussed next (38,46).

Aspergillus sp.

Aspergillus sections are filamentous fungi usually found in soil, decaying vegetation, seeds, and grains, where they thrive as saprophytes (39,47). They can grow in a high range of temperatures and moisture environments (39). *Aspergillus* sp. have a high nutritional versatility and are able to utilize a wide spectrum of organic compounds and a variety of substances can serve as source of carbon (39). Sections from *Aspergillus* genus are found in a wide diversity of indoor environments with different roles, such as decomposers of organic matter (for instance*, Aspergillus* section *Nigri),* mycotoxin producers (*Aspergillus* section *Flavi,* among others), and often as human pathogens from occupational environments (*Aspergillus* section *Fumigati)* (39,46,47)*.*

Important pulmonary pathology is connected with *Aspergillus*-induced allergic and asthmatic lung disease related to occupational exposure (39). The diseases most commonly associated *Aspergillus* are allergic bronchopulmonary aspergillosis, rhinosinusitis, rhinitis, and severe asthma with fungal sensitization (39). Since the development of *Aspergillus* infections depends on the interplay between host susceptibility and the organism, immunocompromised persons are at higher risk and may develop invasive infections (38,39). *Aspergillus* conidia can be found almost everywhere and are easily dispersed in the air (39,47). Since the conidia from *Aspergillus* genus are very small, they are easily inhaled and can colonize the respiratory tract of exposed individuals (39,47).

Aspergillus fumigatus is one of the species most often associated with respiratory symptoms due to the small size of the conidia and other virulence factors associated

with this specific section (39,46,47). However, besides the infections ability, risk assessment should consider the toxigenic potential of species (46). Nevertheless, species or strains belonging to sections *Circumdati, Flavi, Nigri* and *Nidulantes* must be considered in risk assessment of a specific occupational environment, since they include species that produce mycotoxins (39,46).

For the treatment of aspergillosis, the use of triazoles is recommended, but the increase of antifungal resistance has been deserved great concern, due to the potential risk of infections, resulting in difficult clinical management or even death (39,43,46,47). There are two types of resistance: primary and secondary (39,47). A primary (intrinsic) resistance is found naturally among certain *Aspergillus* species without prior exposure to the drug (39). These species have coded in their genome molecular mechanisms that enable them to grow in presence of those antifungals (39). This occurs with *A. fumigatus*, which shows resistance to fluconazole and with some *Aspergillus* cryptic species (i.e., not *sensu stricto*) such as *A. calidoustus*, showing resistance to the triazoles. A secondary resistance (acquired resistance) is the most common and arises when initially susceptible microorganisms develop resistance after exposure of the organism to the drug (39,43). Agricultural fungicides drive acquired drug resistance in *Aspergillus* species, and these resistant strains are spreading globally, which can lead to a global public health threat (39,43).

2.2.2. Bacteria

Bacteria are ubiquitous, comprising an abundance of species (34). They can be found in the dust and on the surfaces of every house, including those with no damp problems (34). The main sources of bacteria in the indoor environment are outdoor air, human and indoor bacterial growth (9,34,37,42,48). Many bacteria that belong to the normal microflora of the human skin, mouth and nose, are continuously emitted into the immediate surroundings of humans, and accumulate indoors (9,42). The number of occupants and their activity were likely to sensitively affect the concentration levels (and compositions) of indoor bacteria (41,42,48). Moreover, insufficient ventilation was also suggested to exert control on the levels of viable bacteria in indoor air (41,42).

As described for fungi, water is a critical requirement for bacterial growth, therefore, in water reservoirs or moist sites of the buildings, bacteria are often found (9,34,37,42). For example, *Legionella* sp. can colonize hot water in the heating systems or even water resulting from the condensation of the cooling systems, living in biofilms that develop on the contact surfaces in the water (9,35,37,42). This can lead to the

Legionnaire's disease, an infection that can result in pneumonia (9,35,37,42). Animals can be important sources, particularly in indoor agricultural environments (37,42).

Bacteria of human origin or commensals are Gram-positive, presenting, in general, no risk to human health (9). Gram-negative bacteria are rare in indoor environments and are generally pathogenic to humans (9). In the outer membrane of Gram-negative bacteria are found endotoxins, which are toxins composed of proteins, lipids and lipopolysaccharides, which can produce a strong immune response, independent on the viability of the bacteria (34,35,41,42,48,49). High levels of exposure to endotoxins can cause respiratory symptoms, but moderate-to-low exposure may protect against allergies and asthma (34,42).

Another major concern is the emergence of antimicrobial resistance that threatens the ability to successfully treat bacterial infections (15,50). For common bacterial infections, high rates of resistance against antibiotics frequently used to treat these infections have been observed world-wide (15).

2.2.3. Viruses

Indoor airborne viruses are most often studied because of concerns about infectious disease transmission (37). Many human pathogenic viruses, such as measles, influenza and norovirus, can spread through the indoor air when they are expelled by infected people or when they are aerosolized by medical procedures, flushing of toilets, and other means (37). In December 2019, a new Coronavirus – *SARS-CoV-2* – was discovered as the causal agent of severe acute respiratory system (16,51). Next, some more characteristics of the *SARS-CoV-2* virus will be presented.

SARS-CoV-2

The virus has rapidly spread internationally, raising global public health concerns, and was subsequently termed coronavirus disease 19 (COVID-19) (16,51). The World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020 (52).

Structurally, *SARS-CoV-2* has four main structural proteins including (53,54):

- Spike (S) glycoprotein, which is a transmembrane protein found in the outer portion of the virus and mediates host cell binding and entry (53,54).
- Nucleocapsid (N) protein, which because is bound to RNA, the protein is involved in processes related to the viral genome, the viral replication cycle, and the cellular response of host cells to viral infections (53). It is also heavily phosphorylated and suggested to lead to structural changes enhancing the affinity for viral RNA (53).

- Membrane (M) glycoprotein, which is the most structurally structured protein and plays a role in determining the shape of the virus envelope (53). It can bind to all other structural proteins (53). Binding with M protein helps to stabilize nucleocapsids or N proteins and promotes completion of viral assembly by stabilizing N protein-RNA complex, inside the internal virion (53).
- Small envelope (E) glycoprotein, which is the smallest protein in the *SARS-CoV* structure that plays a role in the production and maturation of this virus (53).

The most common clinical manifestations of patients with COVID-19 are fever, cough, shortness of breath, and fatigue. Some patients have also shown radiographic ground-glass lung changes and eventually died of acute respiratory distress syndrome (16). By the end of July, there were 188 655 968 confirmed cases of COVID-19, including 4 0067 517 deaths, reported to WHO (55). Their transmission can occur mainly through direct, indirect, or close contact with infected people through infected secretions, which are ousted when an infected person sneezes, coughs, sings or talks (16,51,56,57). In addition, contact with contaminated surfaces is another route of transmission (16,51,56,57). Thus, the virus long persistence on fomites and environmental surfaces is alarming and should be tracked (56). It has been reported that the viability of *SARS-CoV-2* can last in aerosols for about 3 h and this virus can be observed on dry surfaces for 8–72 h, depending on the surface material (56). This virus can also remain on inanimate surfaces infectious for up to 9 days at room temperature (56).

Other environmental factors in buildings, including temperature, humidity, and ventilation and filtering systems, could have a significant influence on the infection (16). COVID-19 has caused serious global damage to public health, community, and the social economy (16). Thus, the *SARS-CoV-2* exposure assessment is critical to implement control measures and guarantee safety of workers from different occupational environments (51).

2.3. Indoor Air Quality in HEI

The HEI represents a unique environment in that it acts as a work environment for faculty, a learning environment for students, and frequently, a home environment for students (6). Thus, they comprise a high population density that spent a long time inside the higher education buildings, which make them susceptible to different types of disease and injuries and this may lead them to absenteeism and delayed the performance of their duties (5–7). Additionally, the HEI are widely varied in their usage, including lecture halls, gyms, dormitories, restaurants, garden, and laboratories (5,7).

Given the magnitude of the population impacted, there is a need to characterize and understand indoor environmental quality in HEI (6). Since many outbreaks of epidemic diseases are correlated with microorganisms in the air (e.g., 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 (58). The assessment of microbiological contamination is an important parameter that contributes to evaluate the air quality in HEI.

2.3.1. Microbiological contamination in HEI

Assessment of microbiological contamination in HEI has been performed in several facilities, such as classrooms, sports hall, laboratories, rooms, entrances, libraries, cafeterias, and restaurants (1,2,4,5,7,18,50,59–63). Each of the above-mentioned studies at universities focused mainly on a single type of environment (64). There is little data on analyzes of fungi and bacteria contamination in multiple indoor environments and assessed the pollutant levels between these environments of various facilities in different HEI (4,7,64). In addition, a recent review analyzed the detection of *SARS-CoV-2* in different environmental samples in different environments, and concluded that the most described indoor environment were health care facilities, followed by waste-water treatment plants, rivers and household, cruises, household environment and industrial occupational environment (51). Although *SARS-CoV-2* detection studies have been carried out so far in at least one school, in HEI environments this has not yet been reported (65).

Entranceways of the building usually have metal door handles that are touched by many students each day, presumably exchanging a portion of their personal skin microbiota with contacted surfaces (5). This is a major concern because it has been shown that pathogenic bacteria can survive on metal surfaces for extended periods of time, even weeks (5). Moreover, *SARS-CoV-2* and fungi were also detected on the door handles (56,62,66). Inadequate personal hygiene can increase the diversity of microorganisms on hands and, by extension, on contacted surfaces (5). This can result in colds and flus that are transmitted, for example, in dormitories and lecture halls (5).

Classrooms are consider one of the main activities of university students, being more crowded and of prolonged use, which have an important influence on the efficiency ant health of students (67). As mentioned before, crowded spaces with many people and close contact favor the proliferation of fungi, bacteria, and *SARS-CoV-2* (2,4,16,60–63). The level of airborne dust is influenced by the type of furniture, equipment, and activities in classrooms (18). Based on previous studies on bioaerosol exposure, fungi

concentration is typically higher in a classroom than in a home, as there are more students inside the classroom (60). Evidence show high percentages of Sick building syndrome symptoms in classrooms (18). For example, laboratories have been widely studied because they are among the rooms that represent the highest levels of microbiological contamination that are related with airborne infections (1,18,60,62,68). Laboratories are used for theoretical and practical classes for students every day and the air can contain a complex mixture of microorganisms, chemicals and contaminants (18,60). The nature of research carried out there is determining (18,60,68). Besides to the presence of harmful pathogens floating in the indoor air, these airborne microorganisms can be deposited on laboratory contact surfaces (62). Laboratory workbenches have been reported to be contaminated by transferring microorganisms from the hands of laboratory technicians to the work surface area during the handling of the microorganisms which they work daily (62). The presence of microbial contaminations in surfaces allows the spread further by the users and could be transferred by other surfaces or person through contact especially when good laboratory practices were compromised (62).

Libraries are places where students spend most of their time and are generally overcrowded places in HEI (2,4,69). Contamination of library collections and premises is of great interest (2,4,69). The amount of microbial content in the indoor air and surface of library has a direct impact on mental health, physical development and performance of the students (69). The library, like any confined space, can house many microorganisms that could not only harm the health of the occupants but also degrade the documentary heritage and wood material of this building (69). Human activity and environmental factors seems to favor fungi and bacteria growth in this places (2,4,69).

Cafeterias are also places with high occupant density (59). These facilities have several activities that favor microbial contamination, such as handling food that may contain a high content of microbes, cooking and poor hygienic processing practices (7,59). Besides that, this are places where people take off the mask to eat and if they are in close contact and the space has poor ventilation, it can promote the aerosol transmission and cause the spread of COVID-19 infection (16).

2.3.2. Sources of microbiological contamination in HEI

In different facilities of universities, several evidences have associated higher levels of bacteria, fungi and *SARS-CoV-2* with periods of large number of people and their activities, increasing in working times (2,4,16,60–63). However, it has been reported that occupation is not the main factor that favor proliferation fungi, since its

concentration decreased as occupation progressed, suggesting that most fungi species presents in the air were not human-borne (1,2). Fungi contamination seems to be favored with dampness of the building, which can be dispersed through droplets during disturbing and then maintained in aerial suspension (2). High concentration of fungi in atmosphere can influence microbiological indoor air contamination (2). For example, before the beginning of the academic year, in indoor air of university rooms were mainly found common outdoor fungi, such as *Cladosporium* sp. and *Alternaria* sp., and its concentration remains stable (2). However, during the academic year a significant variation of fungal genera in the air was observed and concentrations of both fungal genera and other common indoor fungi, such as *Aspergillus* sp., *Penicillium* sp. or *Mucor* sp. was growing steadily during the daytime (2).

In contrast with bacteria, the concentration of filamentous fungi, including *Cladosporium*, was increased by intensive ventilation the air-conditioning operation (63,70). Higher concentration of bacteria and fungi was characteristic of rooms with mechanical ventilation (68). This can due to a lack of or improper cleaning of the mechanical installation, which can act as a source for microbial proliferation and spreading of microorganisms (68). Obviously, the presence of a good ventilation system inside buildings eliminates to some extent the influence of indoor sources(2,4). Furthermore, good ventilation is one of the recommended measures to prevent spread of *SARS-CoV-2* (16).

The season is also a factor that influence the microbiological contamination in HEI (1,63). There are been suggested that in the warmer season higher indoor microbial concentration are found (1). For example, one study reported that in winter with cold with snow, the surrounding surfaces including the area of air inlet were covered with snow and clean, without a source of fungi (63). Inside of lecture room there were obtained very low concentrations of all kinds of fungi (63). However, the transition of seasons changes the indoor microenvironment and affects other factors such as opening windows, heating method, operation of air conditioning, sunlight, humidity, mold in walls, as well as outdoor microorganisms, which together lead to changes in microbial concentrations (71). Therefore, only reporting the season in field measurement is not enough to fully understand the change of concentration(71).

The lack of bathrooms cleaning regularly, unused effective detergents and antiseptic and inappropriate cleaning mode are pointed out as source of contaminated and spread of microbes in different facilities (7). Besides that, effective cleaning of surfaces can reduce the risk of contact transmission of *SARS-CoV-2* (56).

2.4. Legal and Scientific Guidelines

For exposure levels of indoor bacteria and fungi, different countries or organizations have different standards (71). There is a clear need to globally standardize regulations to assess the risk of occupational exposure based on health effects (71).

In Portugal, in recent years there has been an increased concern with indoor air as a vehicle of pollutants and contaminants (72). Thus, recently a specific law was published (73) in order to standardize protection thresholds and reference conditions related to IAQ. With regard to current Portuguese legislation, limit values for microbiological contamination are established for indoor environments in general, without any specification for education environments (73). The present Portuguese legal compliance varies concerning the microorganisms (73).

Regarding to surfaces, there is no legal reference mentioning the assessment of bioburden (24). However, several studies indicate that in the case of fungal contamination on surfaces there is the possibility of re-aerosolization during the dispersion of fungal spores, varying with fungal characteristics and environmental variables (21,24). In addition, evidence suggests that contamination of surfaces by bacteria contributes to a 15% increase in airborne contamination (24). Furthermore, for *SARS-CoV-2* detection, surface swabs has been widely used, corroborating their importance in the assessment of bioburden exposure (comprising fungi and bacteria) in indoor environments (51).

2.5. Methodology for sampling and microbiological characterization

To assess microbiological contamination active and passive sampling methods can be used. For microbiological characterization culture-based methods and molecular tools are used. Next, will be explained these methods.

2.5.1. Active and passive sampling

Exposure assessment to microorganisms remains a challenge to every exposure assessor (39). Occupational exposure to microbiological risks can be estimated using a variety of different methods and each situation is unique and requires specific methodology (74). The microbiological content of the air can be monitored by active and passive sampling methods, which encompass various techniques (75,76).

Active methods can only reflect the load from a shorter period of time (mostly minutes), corresponding to the sampling duration and representing only a small fraction of the bioburden exposure (24,39,46,51,77,78). In contrast to passive methods, active air

sampling techniques require specialized equipment and trained staff to operate the equipment (79). In active methods microbiological air sampler physically draws a known volume of air through or over a particle collection device which can be a liquid (impinger) or a solid culture media (impaction) or a membrane filter (filtration) and the quantity of microorganisms present is measured in CFU (colony-forming units)/m^3 of air (20,74,76,80). This method is recommended when the concentration of microorganisms is not very high (76,78). It can also be used to obtain information on the concentration of inhalable airborne particles in indoor environments (78).

Passive methods allow determination of the contamination levels from a larger period of time (days, weeks or several months) (24,39,46,77,78). It provides a valid risk assessment, since it permits measurement of the harmful part of the airborne population which falls onto a critical surface (74,75,78). It is a readily available, economic, and unobtrusive method (75). Passive methods mainly include surface swabs, electrostatic dust cloth (EDC) and settle plates (37,39,46,74,76).

As described above, both methods have advantages and disadvantages, and the choice of appropriate sampling method is mainly determined by the objectives of the study (80). The main difference between them is the period of time that reflects the levels of contamination, with passive methods reflecting a larger period of time (24,39,46,77,78). Besides that, in passive method, the microorganisms that are collected at the time of sampling are unable to capture those that are still floating in the air and take more time to fall on surfaces (81). The spread in a bio-aerosol is heterogeneous, so whatever is ‘catched’ on that moment may vary from the second, third or even fourth sampling attempt (81).

Several studies have been recommended that passive and active methods should be used in parallel to ensure a more precise assessment of occupational exposure to bioburden (51,74,77). Increasing the number of different sampling methods will enrich data findings, enabling industrial hygienists to perform risk characterization (74,77).

2.5.2. Culture-based methods and molecular methodologies

Although culture-based methods is widely used to characterize indoor microbial communities, molecular methods have also emerged in recent years (37).

Culture-based methods determine if the microorganisms are viable and thus capable of growth and infection (37). These methods are relatively inexpensive, well developed, quantitative, taxon specific and allow an easy quantification of culturable microorganisms with unaided eye (48,82,83). Furthermore, azole resistance screening can only be performed by culture-based methods (24,84). They have a high sensitivity

with appropriate media and provide the identification of different species (12,82,83). However, these techniques can be difficult and time-consuming (37,80,83). Only viable organisms are measured and only a subset of airborne organisms is culturable (48). These methods are selective, revealing only microorganisms able to grow on a particular growth media, therefore, underestimating the total number of microorganisms in samples (24,37,80,85). In addition, the vast majority of microorganisms currently cannot be cultivated by routine laboratory methods, and many microorganisms, can enter a viable but non-culturable state in which they cannot be readily cultured in the lab (37). Besides the viable part constitutes only between 1 and 25% of total bioburden, probably represent higher health risks when compared with non-viable organisms (24,84). The non-viable and non-cultivable portion of bioaerosols can still be harmful to exposed persons since several health issues related to bioaerosol exposure are not linked to microorganism viability or infectious potential (37,80). The most tolerant and fast-growing species can inhibiting others, with clinical relevance, to grow (37).

Molecular methods offer a broader view of the microbial diversity when compared with culture-based methods (37,80). Thus, allow to overcome some culture-based methods constraints (39). They are sensitive, specific, and reduced processing time (80). One of the most common and recommended molecular method for the detection of microorganisms in environmental samples is the PCR (polymerase chain reaction) assay, more specifically real-time quantitative PCR (qPCR) (80). qPCR permits to quantify the abundance and expression of taxonomic and functional gene markers in various environmental samples. The American Industrial Hygiene Association indicates qPCR to detect harmful fungal contamination, as an established and very sensitive molecular technique, which has shown its potential to detect pathogens in many fields, leading to earlier diagnosis and improved patient outcome (86). However, all molecular methods also have disadvantages such as, for example, the effectiveness of DNA extraction, PCR biases or the short size of the amplified DNA fragments which do not allow the identification at the species level (39,85). Limitations of qPCR include ineffective release of microbial DNA content from cells, or poor DNA recovery after extraction and purification steps, or to the presence of exogenous substances, called inhibitors, namely the presence of particles in the air as previously reported (87–89). Besides that, qPCR is more expensive than conventional approaches (88).

Based on the advantages and disadvantages of culture-based methods and molecular methods, the use of both methods seems to be the best way to provide a fuller image of indoor bioaerosols and o overcome the limitations of each one (37,39,80,89).

3. Methodology

This chapter describes the methodology involved in carrying out the project, including the characterization of the study site and the procedures.

3.1. Characterization of the study site

This study was performed between July of 2020 and July of 2021 in ten selected units of Portuguese HEI, including the presidency building, social services and eight faculties. It was part of an enlarged study with financial support to implement an integrated approach on Occupational Health to tackle COVID-19 pandemic and microbiological contamination assessment, in order to ensure safety in academic recovery during the pandemic crises (90). Despite all these analyzes, this study focused on microbiological contamination assessment (comprising SARS-CoV-2, fungi, and bacteria).

It was a cross-sectional study, observational, as it intends to characterize the microbiological contamination existing in HEI under study in a specific period of time in which the sampling is carried out and describe its importance for public health.

3.1.1. Localization of the study site

The HEI under study are located in Lisbon district, as described in Figure 3.1.

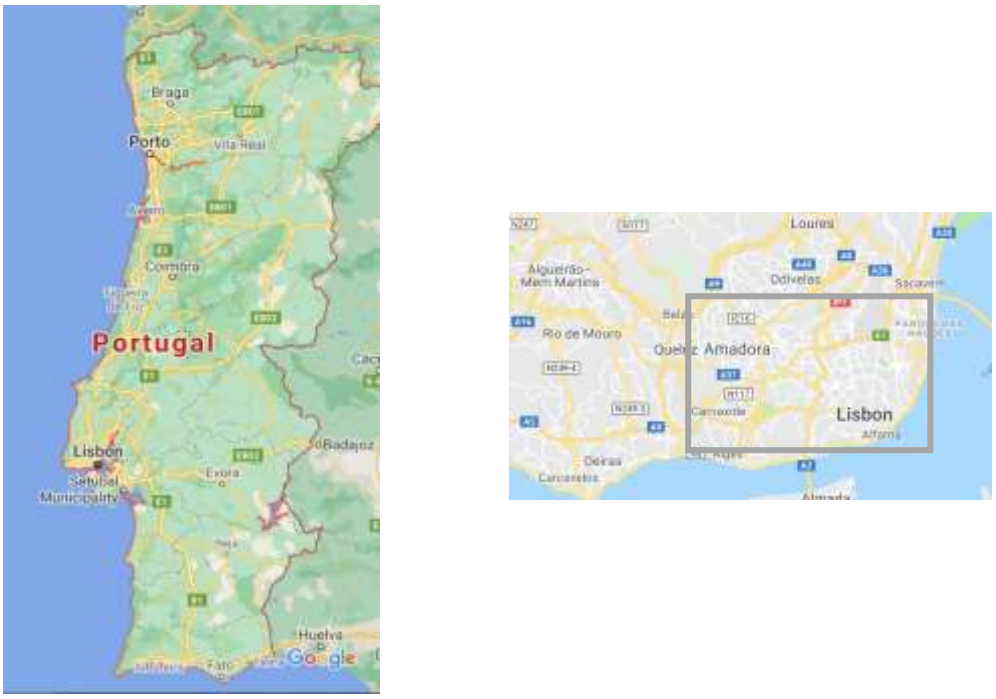


Figure 3.1 – Localization of the study site (adopted from (140))

In order to ensure anonymity and confidentiality, neither the name of the site nor any indication that makes it possible to understand their location will be made available. Therefore, HEI will hereinafter be designated as HEI 1, HEI 2, HEI 3, HEI 4, HEI 5, HEI 6, HEI 7 and HEI 8. The presidency building and social services will be defined as PB and SS, respectively.

Table 3.1 shows some characteristics related to each study site, such as the developed activities depending on their purpose and graduation courses held and the building exterior features.

Table 3.1 – Characteristics related to each study site (adopted from (91)).

Study site	Developed activities	Building exterior features
PB	Presidency services that support the HEI in the activities common to the institution	Urban area (next to high road and rail traffic routes)
SS	Social services that implement the school social action policy and the provision of support and benefits to students who attend the Institution, oriented to the improvement of study conditions	Urban area (next to high road and rail traffic routes)
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	Urban area (next to road and rail traffic routes)
HEI 2	Accounting and business technicians to perform administrative and financial accounting activities of national organizations	Urban area (next to high road traffic routes)
HEI 3	Classic engineering areas and in the area of renewable energies, acoustics, environment, hygiene and safety, urban rehabilitation, biomedical engineering and management	Urban area (next to road traffic routes)
HEI 4	Theater and Cinema, including conducting research activities and experimentation and artistic production	Urban area (residential, next to road traffic routes and a city park)
HEI 5	Training of teachers, and other educational agents with a high level of cultural, scientific, technical, and professional preparation	Urban area (next to high road and rail traffic routes)
HEI 6	Artistic, technical, technological, and scientific training for music professionals	Urban area (next to high road and rail traffic routes)
HEI 7	Social communication, including the areas of Audiovisual and Multimedia, Journalism, Advertising and Marketing, and Public Relations and Business Communication	Urban area (next to high road and rail traffic routes)
HEI 8	Higher education in Dance	Urban area (next to road traffic routes)

3.1.2. Sampling site selection

The sampling site were previously selected in different areas of each study site by the Occupational Health Services in the context of the detection of *SARS-CoV-2*. To this end, a walkthrough survey and checklist were applied in order to prioritize the most critical workplaces/areas in relation to *SARS-CoV-2* contamination and also in order to

reflect the variability of the environment. Thus, areas of each study site were selected according to the following criteria: 50-80% workplaces/facilities occupation, activities performed or based on workers positive serologic surveillance results (positive result for IgM+ or IgG +) (90).

Environmental samples (air and surface samples) were performed in each area considering the criteria described above. Between 6 and 25 samples were collected at each location (average of 8 samples), including an average of 5 air samples for determining *SARS-CoV-2* and fungi; 11 surface swabs for detection of *SARS-CoV-2*; and 9 surface swabs for the determination of fungi and bacteria (Table 3.2) (91).

Several areas were sampled at each study site, but most of them were common among all, such as offices (including human and financial resources, academic services, logistics, accounting, and acquisition department), attendance rooms, reception rooms, auditoriums, meals spaces, bathrooms, and libraries. Although there are classrooms in almost every location, the type of classroom analyzed varies widely in all locations, including rooms for music, dance, choir, theater and multimedia, laboratories, and gyms. In addition, in some locations, there were samples collected from student's social rooms, workshops, changing rooms and professor's room (Table 3.2) (91).

In each sampling site, some information was recorded that could have an impact on the presence of microorganisms, such as the quantification of occupants, the date and time of sampling, the sampling surface material, the cleaning method, the cleaning products used and the cleaning frequency. Sampling was carried out between summer and autumn. The samples were collected mainly in the morning and during normal activities, except for HEI 1, which was carried out in two days, one of them in the afternoon. In all facilities, the cleaning method was cleaning, and disinfection and the cleaning products used were bactericide and virucide, bleach and multipurpose detergent. Most of the sampling sites (56.8%) registered between 1 to 9 workers, in the facilities during the samples collection (91).

Table 3.2 described below show the number of air and surface samples collected in the sampled areas at each study site.

Table 3.2 - Number of surface swabs and air samples collected in the sampled areas at each study site (adopted from (91)).

Study site	Assessed areas	Surface swabs (for *SARS-CoV-2*)	Surface Swabs (for fungi and bacteria)	Air samples
PB	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	
SS	Lunchroom (Table)	0	1	1

	Lunchroom (Microwave, Refrigerator)	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	1
	Reception (Door and Water Machine)	1	1	
	Bathroom (Doors and Faucets)	1	1	1
	Bathroom (Floor)	0	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	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	Financial Services (Printers and Stapler)	1	1	1
	Professor’s Room 1 (Printers and Computers)	1	1	1
	Professor’s 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	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
HEI 4	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	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	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	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	
HEI 8	Academic Services (Printer, Doorknob, Acrylic and Table)	1	1	0*
	Dance Reception (Doorknob, Computer, Telephone and Keys)	1	1	
	Professor's 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 sampling device was not operational due to technical constrains.

3.2. Procedures

The procedures performed, including sampling, laboratory methodologies used for detection of *SARS-CoV-2* and fungal and bacterial characterization, and statistical analysis are described below.

3.2.1. Sampling Methodology

The two sampling methods applied, and the materials and equipment used are described below.

Active sampling methods

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 3.2) (90,91).

Coriolis µ is a portable biological air sampler for bio-contamination assessment (92). It is based on a cyclonic technology, combined with a high air flow rate (92). These samplers work by drawing air through liquid causing the airborne particles to become suspended (93). Biological particles such as toxins, viruses, bacteria, molds, pollens

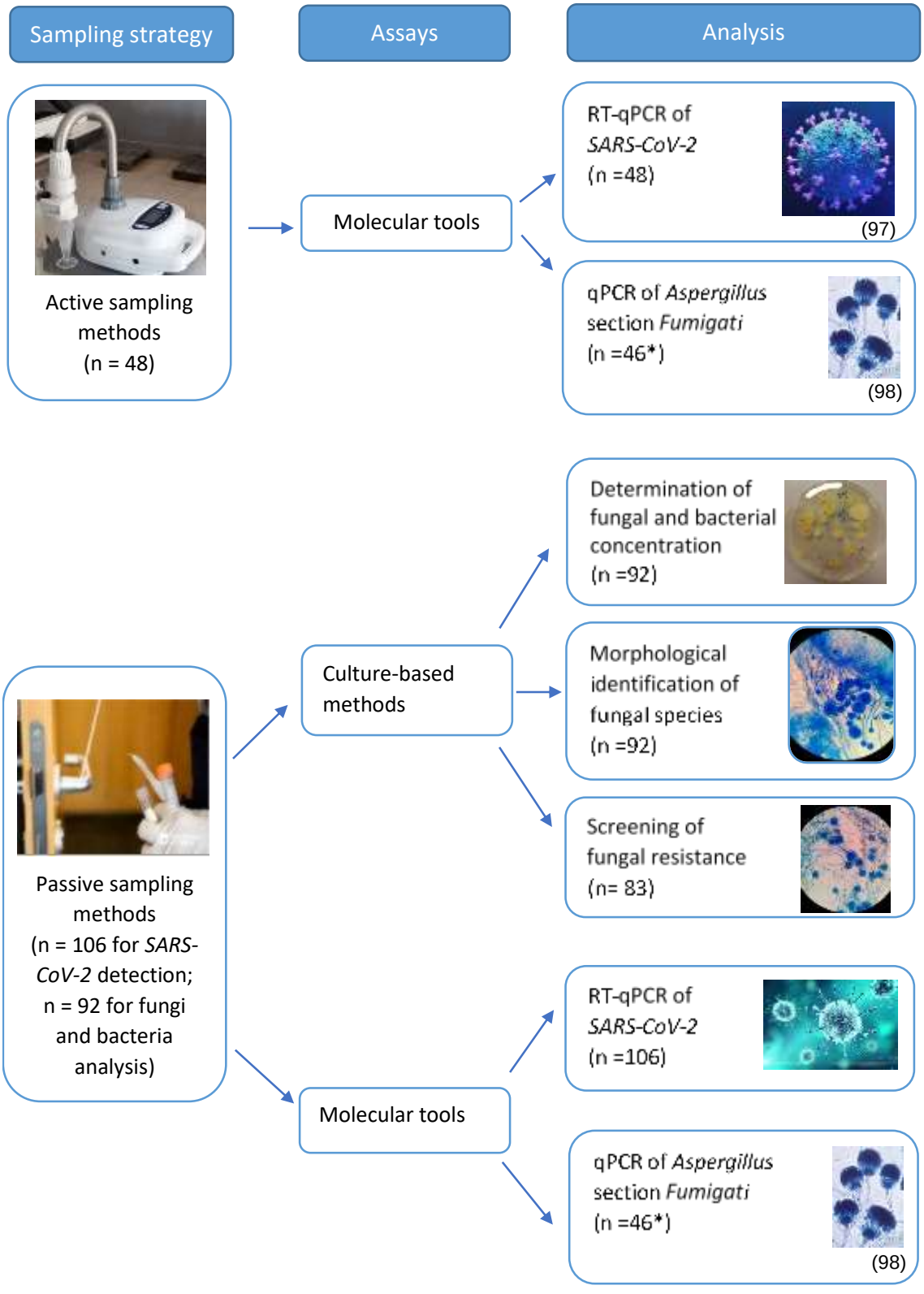
and spores are collected and concentrated in a liquid ready to be analyzed with microbiological and cellular and molecular biology methods (92).

The Buffer NVL is a denaturing lysis buffer containing guanidine thiocyanate, which also inactivates cellular RNases, to ensure the recovery of intact RNA molecules (94).

Passive sampling methods

Surface samples were collected by swabbing the areas of each sampling site, using sterile cotton swabs moistened in sterilized water. A 10 cm x 10 cm square stencil, disinfected between samplings with a 70% alcohol solution was used (95) to allow quantification. On some surfaces with common characteristics, such as the type of use, surfaces material and cleaning procedures, composite samples were performed (swabbing different surfaces with the same swab) (90,91,96) (Figure 3.2).

Then, for *SARS-CoV-2* detection, the two sterile cotton swabs collected for each accessed area were stored into a 15 mL falcon containing 1.5 mL Buffer NVL (NZY Viral RNA Isolation kit (MB40701) component). On the other hand, for the analysis of fungi and bacteria, one sterile cotton swab per sampled area was used, which was previously moistened in sterilized water and after the sampling was placed in the appropriate swab tube (90,91) (Figure 3.2).



* Lack of extracts quantities in 2 samples to perform the assay

Figure 3.2 - Sampling plan (adopted from ((91))).

3.2.2. Characterization of fungal and bacterial contamination

Fungal and bacterial contamination was characterized by culture-based methods. However, fungi were also characterized through molecular tools. These methodologies are mentioned below.

Culture-based methods

In culture-based methods surface samples were only used. First it was necessary extract the samples from swabs 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 Buhler SM-30, Hechingen, Germany) (91).

Then, to enhance the selectivity for bacterial and fungal growth, 150µL of the samples extracted from surfaces swabs were inoculated onto four different culture media: 2% malt extract agar (MEA) supplemented with 0.05 g L-1 chloramphenicol media were used for fungi, dichloran glycerol agar (DG18) agar based media were used for xerophilic fungi, tryptic soy agar (TSA) supplemented with 0.2% nystatin to assess total bacterial, and violet red bile agar (VRBA) to assess coliforms (Gram-negative bacteria). Antifungal resistance in all surface samples (except for PB samples due to the lack of samples) was also screened by inoculating 150µL of the samples on Sabouraud dextrose agar (SAB) supplemented with 4 mg/L itraconazole (ITR), 2 mg/L voriconazole (VOR), 0.5 mg/L Posaconazole (POS), or SAB alone (as control) (adapted from (99)) (91).

The characteristics of each culture media used are described on Appendix 8.1 – Description of culture media used.

All the inoculated plates were incubated at 27°C for five days for fungal growth 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 (CFU/m^2) results for fungi and bacteria were obtained (91). To obtain the CFU per measuring unit was used this formula:

$$CFU.m^{-2} = CFU \times 1000, \text{ where CFU are the colonies counted}$$

When colony overgrowth was observed due to fungi and bacteria with fast growing rates (Mucorales group, *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 (91).

Fungal species were also identified microscopically using lactophenol cotton blue mount procedures. Morphological identification was achieved through macro and microscopic characteristics (91,100).

Molecular tools

Air samples obtained by impinger and surface swabs were used for the molecular detection of a fungal specie with reported toxigenic potential, namely *Aspergillus* section *Fumigati*. Fungal DNA was extracted from the samples mentioned above using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA), according to the manufacturer's instructions (91).

Molecular identification of the different fungal species/strains was obtained by qPCR, using the automation system (CFX)-Connect PCR System (Bio-Rad, California, USA). Reactions included 1 x iQ Supermix (Bio-Rad, California, USA), 0.5 µM of each primer (Table 3.3), and 0.375 µM of TaqMan probe in a total volume of 20 µL. Amplification followed a three-step PCR: 50 cycles with denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s. A non-template control was used in every PCR reaction. As positive controls of amplification, DNA samples were obtained from reference strains, belonged to the culture collection of the Reference Unit for Parasitic and Fungal Infections, Department of Infectious Diseases of National Institute of Health Dr. Ricardo Jorge the Mycology Laboratory. These strains have been sequenced for Internal transcribed spacer (ITS), B-tubulin, and Calmodulin.

Whenever a sample showed dubious results, this was repeated. The results were classified as positive or negative for the detection of *Aspergillus* section *Fumigati* and the Cq (Quantification cycle) value was registered.

Table 3.3 shows the sequence of primers and TaqMan probes used for qPCR detection of *Aspergillus* section *Fumigati*.

Table 3.3 - Sequence of primers and TaqMan probes used for qPCR (adopted from (101)).

	Sequences	**Reference**
Fungal Species	***Fumigati***	
Forward Primer	5‘–CGCGTCCGGTCCTCG–3‘	
Reverse Primer	5‘–TTAGAAAAATAAAGTTGGGTGTCGG–3‘	(102)
Probe	5‘–TGTCACCTGCTCTGTAGGCCCG–3‘	

3.2.3. Detection of *SARS-CoV-2*

For the detection of *SARS-CoV-2* it was necessary to follow the steps described in the Figure 3.3 which are in detail bellow.

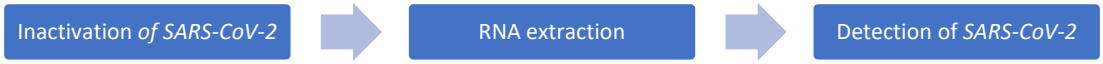


Figure 3.3 – *SARS-CoV-2* detection protocol.

Inactivation of *SARS-CoV-2*

In the laboratory, the vials of Coriolis and the swabs containing the samples for the detection of *SARS-CoV-2* were externally disinfected with 1% sodium hypochlorite solution. Then, the vials and the swabs were placed in water bath at 56ºC for 30 minutes.

RNA extraction

RNA was extracted from the samples collected (5 mL in air samples and 1.5 ml in surface samples) with the NZY Viral RNA Isolation kit, from Nzytech, according to manufacturer's instructions (90).

Detection of *SARS-CoV-2*

The detection of *SARS-CoV-2* were carried out through the molecular tool RT-qPCR using the CFXConnect PCR System (Bio-Rad). Reactions included 1 x Master Mix (NZYSpeedy One-step RT-qPCR probe Master Mix) with 400 nM of each primer and 100 nM of TaqMan probes published by CDC (available on (103)), which detected two viral gene regions (N1 and N2) (Table 3.4). Total volume of reactions was 10µL (90).

In each analysis a positive (a *SARS-CoV-2* positive sample, obtained from Chronic Diseases Research Centre - Universidade NOVA de Lisboa, and from Instituto de Medicina Molecular João Lobo Antunes) and a negative (water) sample was included. Besides that, an internal control (TATAA Universal RNA Spike I) was added to assess potential inhibitors present in the samples collected (90) (Table 3.4).

Amplification followed a three-step PCR: 45 cycles with denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 30 s.

All the results are analyzed by two independent researchers. Whenever a sample showed dubious results, including, for example, lack of amplification of both viral gene regions, they were considered inconclusive, and the assay was repeated. The results were classified as positive or negative for the detection of *SARS-CoV-2* (90).

Table 3.4 shows the panel primers and probes used.

Table 3.4 - Novel Coronavirus (2019-nCoV) Real-time RT-qPCR Panel Primers and Probes (adopted from (90)).

Name	Description	Oligonucleotide Sequence (5'>3')	Label1
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC AGC GCT TTC AG-BHQ1-3'	FAM, BHQ-1
Spike In		TATAA Universal RNA Spike I	FAM

3.2.4. 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 (91).

4. Results

This chapter presents the results regarding bacterial and fungal contamination and detection of *SARS-CoV-2*, which are submitted for publication (90,91).

4.1. Bacterial contamination by culture-based methods

Surface samples showed a total bacterial contamination of 9.7 x 10^6 CFU.m-^{2}, ranging between 1 x 10^3 CFU.m^{-2} (classroom and attendance room) and 3.1 x 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 x 10^5 CFU.m^{-2} (study room) and the lowest was 1 x 10^3 CFU.m^{-2} (classroom and attendance room). Gram-Negative bacteria in surface samples was 7.1 x 10^4 CFU.m^{-2}, ranging from 0 to 1.9 x 10^4 CFU.m^{-2} (laboratory) with a highest median value of 9.5 x 10^3 CFU.m^{-2} in the laboratory (Figure 4.1 and Figure 4.2).

Total bacterial contamination found in different facilities ranged from 3.8 x 10^4 CFU.m^{-2} (HEI 2) to 4 x 10^6 CFU.m^{-2} (HEI 7), with the highest median value found in HEI 7 (5 x 10^5 CFU.m^{-2}) and the lowest median value found in SS (2 x 10^3 CFU.m^{-2}). Gram-negative bacteria ranged between 0 and 3 x 10^4 CFU.m^{-2} (HEI 3), with a highest median value of 4 x 10^3 CFU.m^{-2} (HEI 3) (Figure 4.1 and Figure 4.2).

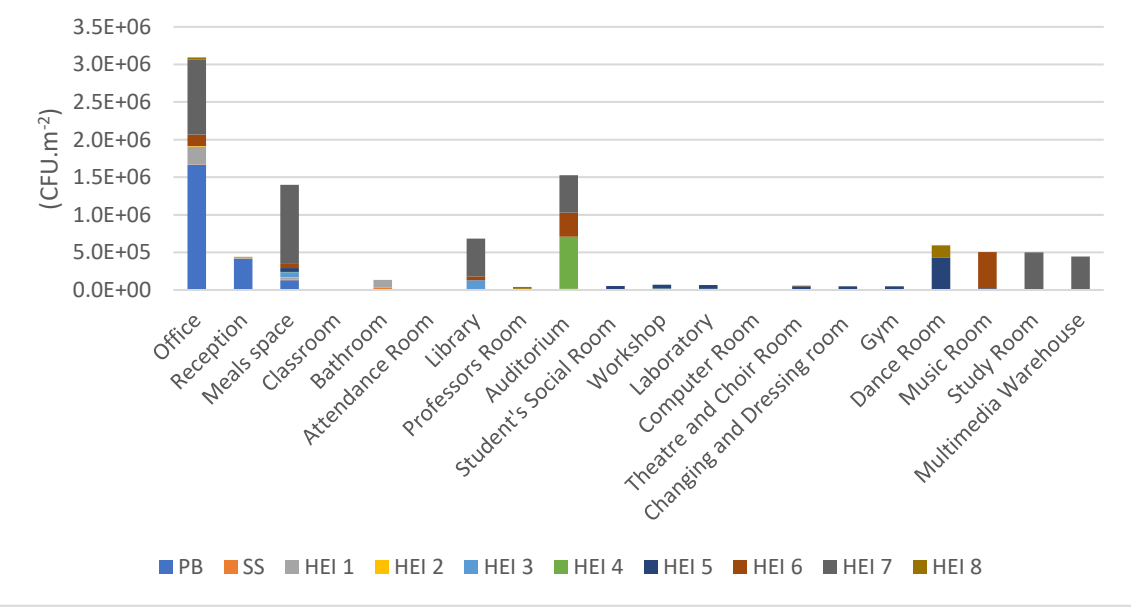


Figure 4.1 – Total bacteria in surface swabs (adopted from (91)).

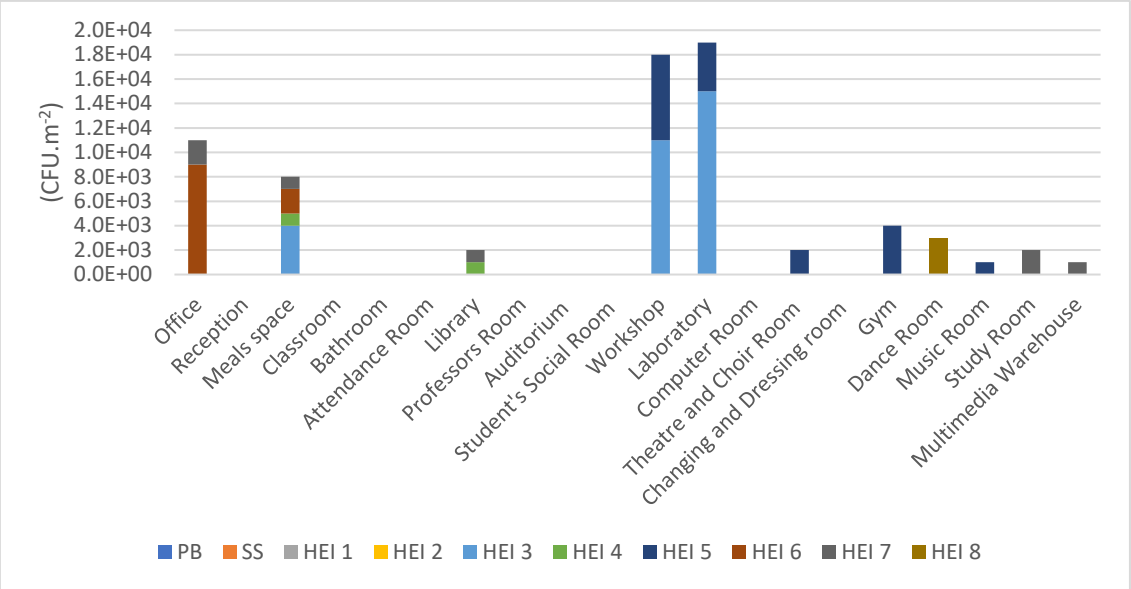


Figure 4.2 – Gram-negative bacteria in surface swabs (adopted from (91)).

4.2. Fungal contamination by culture-based methods

The total fungal contamination on the surfaces ranged from 0 to 1.5 x 10^5 CFU.m^{-2} (offices) on MEA, and from 0 to 1.8 × 10^5 CFU.m^{-2} (professor's room) on DG18, totaling 5.3 x 10^5 CFU.m^{-2} on MEA and 5.7 x 10^5 CFU.m^{-2} 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} (professor's room) in DG18 (Figure 4.3 and Figure 4.4).

Fungal contamination found in different facilities ranged from 2 x 10^3 CFU.m^{-2} (HEI 4) to 1.8 x 10^5 CFU.m^{-2} (HEI 6) on MEA, and from 5 x 10^3 CFU.m^{-2} (HEI 4) to 1.7 x 10^5 CFU.m^{-2} (HEI 2) on DG18. The median values on MEA ranged between 0 (HEI 4) to 3.1 x 10^4 CFU.m^{-2} (HEI 6), and from 0 (HEI 4) to 8 x 10^3 CFU.m^{-2} (HEI 6) on DG18 (Figure 4.3 and Figure 4.4).

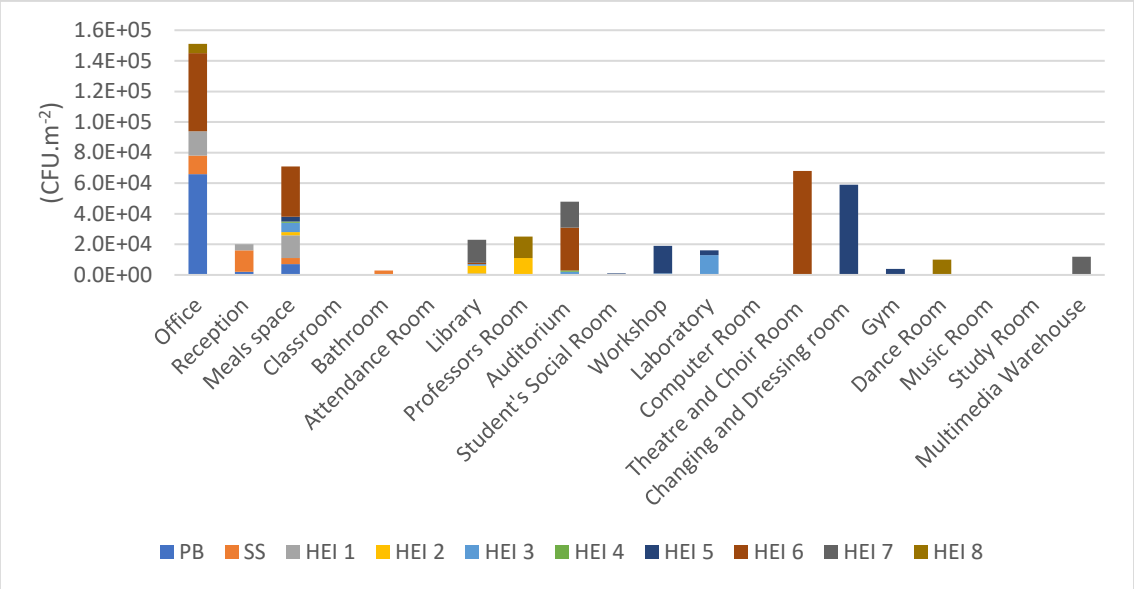


Figure 4.3 – Fungal contamination in surface swabs on MEA (adopted from (91)).

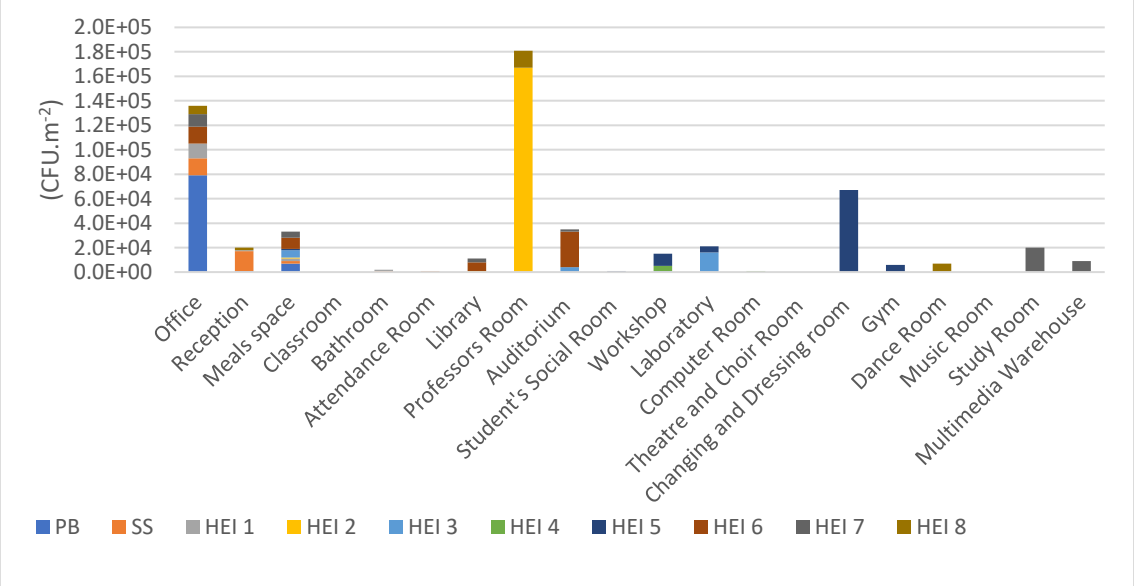


Figure 4.4 – Fungal contamination in surface swabs on DG18 (adopted from (91)).

Regarding fungal distribution, 13 different fungal species were observed, being found in total 10 and 9 on MEA and DG18, respectively. The most prevalent species found on both media were *Cladosporium* sp. (47.36% MEA; 32.33% DG18). Other common species observed on MEA were *Penicillium* sp. (40.94%) and *Aspergillus* sp. (3.21%), whereas on DG18 were *Aureobasidium* sp. (28.97%) and *Penicillium* sp. (20.67%) (Table 4.1).

Table 4.1 – Fungal species distribution in surface swabs after inoculation onto MEA and DG18 media (adopted from (91)).

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

Aspergillus genera was observed on MEA (3.21%) and DG18 (14.66%), with 6 different species identified on both media (Table 4.1). There were found 5 different *Aspergillus* sections on MEA, being *Candidi* the most prevalent (6 x 10^3 CFU.m^{-2}; 35.29%), followed by *Fumigati* (5 x 10^3 CFU.m^{-2}; 29.41%), *Nigri* (3 x 10^3 CFU.m^{-2}; 17.65%), *Nidulantes* (2 x 10^3 CFU.m^{-2}; 11.76%) and *Aspergilli* (1 x 10^3 CFU.m^{-2}; 5.88%). On DG18 there were found 3 different *Aspergillus* sections, being *Nidulantes* the most identified (7.7 x 10^4 CFU.m^{-2}; 92.77%) followed by *Candidi* (5 x 10^3 CFU.m^{-2}; 6.02%) and *Circumdati* (1 x 10^3 CFU.m^{-2}; 1.20%) (Figure 4.5).

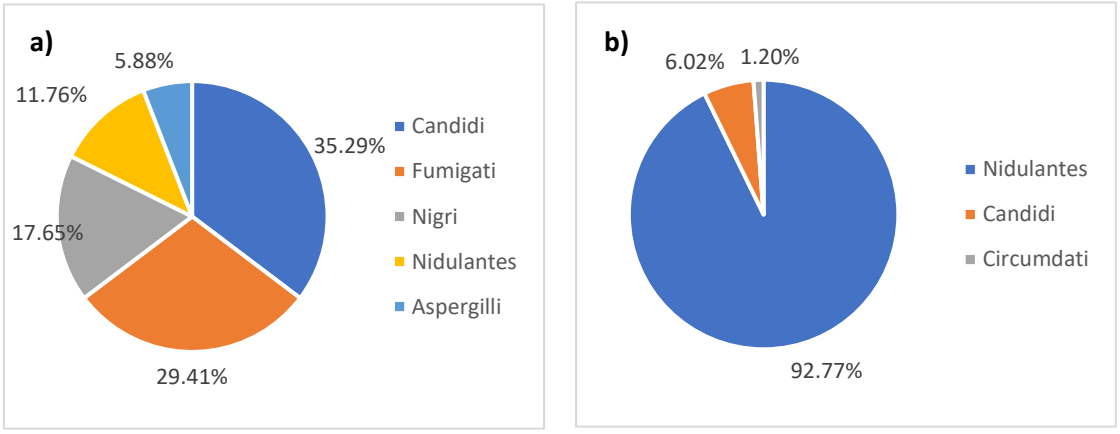


Figure 4.5 – *Aspergillus* sections distribution in surface swabs after inoculation onto a) MEA and b) DG18 (adopted from (91)).

4.3. Azole resistance screening

Forty-seven of the eighty-three samples (56.63%) showed the growth of ten distinct fungal species on SAB supplemented with azoles. Eighteen of the eighty-three samples (21.69%) presented growth of six fungal species on 4 mg/L ITR (*Cladosporium* sp., *Chrysosporium* sp., *Chrysonilia sitophila*, *Alternaria* sp., *Mucor* sp., *Penicillium* sp.); thirty-six of the eighty-three samples (43.37%) presented growth of nine fungal genera on 2 mg/L VOR (*Cladosporium* sp., *Penicillium* sp., *Chrysosporium* sp., *Aureobasidium* sp., *Chrysonilia sitophila*, *Mucor* sp., *Acremonium* sp., *Alternaria* sp., *Aspergillus* section *Nigri*); twenty of the eighty-three samples (24.10%) presented growth of six fungal genera on 0.5 mg/L POS (*Cladosporium* sp., *Penicillium* sp., *Chrysosporium* sp., *Chrysonilia sitophila*, *Mucor* sp., *Rhizopus* sp.) (Table 4.2).

Table 4.2 – Fungal distribution in azole-supplemented SAB media from surface swab samples (adopted from (91)).

	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 x 10^4; 3.44	0.00	1.00 x 10^3; 0.70	0.00
Alternaria sp.	1.00 x 10^3; 0.31	2.00 x 10^3; 3.92	1.00 x 10^3; 0.70	0.00
Aspergillus section *Candidi*	2.00 x 10^3; 0.63	0.00	0.00	0.00
Aspergillus section *Fumigati*	3.00 x 10^3; 0.94	0.00	0.00	0.00
Aspergillus section *Nidulantes*	5.00 x 10^3; 1.56	0.00	0.00	0.00
Aspergillus section *Nigri*	8.00 x 10^3; 2.50	0.00	1.00 x 10^3; 0.70	0.00
Aspergillus section *Circumdati*	1.00 x 10^3; 0.31	0.00	0.00	0.00
Aureobasidium sp.	9.00 x 10^3; 2.81	0.00	6.00 x 10^3; 4.23	0.00
Bipolaris sp.	3.00 x 10^3; 0.94	0.00	0.00	0.00
Chrysonilia sitophila	1.00 x 10^3; 0.31	3.00 x 10^3; 5.88	4.00 x 10^3; 2.82	1.00 x 10^3; 2.22
Chrysosporium sp.	2.20 x 10^4; 6.88	9.00 x 10^3; 17.65	1.30 x 10^4; 9.15	8.00 x 10^3; 17.78
Cladosporium sp.	1.93 x 10^5; 60.31	3.30 x 10^4; 64.71	9.30 x 10^4; 65.49	2.40 x 10^4; 53.33
Mucor sp.	1.30 x 10^4; 4.06	2.00 x 10^3; 3.92	2.00 x 10^3; 1.41	1.00 x 10^3; 2.22
Penicillium sp.	4.40x10^4; 13.75	2.00 x 10^3; 3.92	2.10 x 10^4; 14.79	1.00 x 10^4; 22.22
Rhizopus sp.	3.00 x 10^3; 0.94	0.00	0.00	1.00 x 10^3; 2.22
Ulocladium sp.	1.00 x 10^3; 0.31	0.00	0.00	0.00
Total	3.20 x 10^5; 100	5.10 x 10^4; 100	1.42 x 10^5; 100	4.50 x 10^4; 100

4.4. Molecular detection of *Aspergillus* section *Fumigati*

In all the air samples *Aspergillus* section *Fumigati* was not identified by the molecular tool qPCR. However, this section was detected in 2 surface samples (2.22%, 2 out of 90 samples). Interestingly, the presence of this section was not identified by culture-based methods in any of the samples collected (Table 4.3).

Table 4.3 – Molecular detection of *Aspergillus* section *Fumigati* (adopted from (91)).

***Aspergillus* section detected**	**Sample type**	**CFU.m^{-2} (in MEA/DG18)**	**C_q**
Fumigati	Swab	0/0	31.56
		0/0	28.32

4.5. Molecular detection of *SARS-COV-2*

In all the environmental samples analyzed, comprising air and swabs samples, the results were negative regarding *SARS-CoV-2* detection. In all PCR experiments positive control amplified, and internal control also amplified and at the same CT value for all the samples and controls, indicating no PCR inhibition in the collected samples.

4.6. 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 4.4).

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 4.4)

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* section, 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* section in MEA and higher number of workers (Table 4.4).

With respect to fungal counts in DG18, significant correlations were detected 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* section 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* section in DG18 (Table 4.4).

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* section – azole resistance in SAB (rS= 0.388, p=0.000), showing that higher counts in SAB is related to greater resistance to azoles in other media, including in the *Aspergillus* section (Table 4.4).

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 4.4).

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* section in MEA (rS= 0.243, p=0.027) and *Aspergillus* section 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* section in MEA and higher and azole resistance in Aspergillus section in SAB (Table 4.4).

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) azole resistance 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 4.4).

Table 4.4 - Study of the relationship between bacterial and fungal counts, azole resistance (SAB, ITR, VOR and POS) and *Aspergillus* section (fungi (MEA, DG18) and azole resistance (SAB, VOR). Spearman correlation coefficient results (adopted from (91)).

			Bacterial counts ($CFU.m^{-2}$)	Fungal counts ($CFU.m^{-2}$)		Azole resistance ($CFU.m^{-2}$)				*Aspergillus* section				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
	Fungi	MEA									0.068	0.010	0.360**	0.189
Aspergillus		DG18										-0.083	-0.025	-0.027
section	Azole	SAB											0.294**	-0.072
	resistance	VOR												0.190

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

5. Discussion

The discussion of the results presented below is reported in previously submitted publications (90,91).

The IAQ in HEI is of great importance due to the impact it has on the health and performance of students, professors and other workers (5–7). Fungi and bacteria have been widely studied for their negative health effects (9–11). However, in the past year, the emergence of the *SARS-CoV-2* virus has caused extensive damage around the world, emphasizing the need to analyze the presence of microorganisms in indoor air (16). Since potentially pathogenic microorganisms can be disseminated as bioaerosols and via contact with contaminated surfaces, microbiological contamination assessment is one of the main parameters that affect IAQ (3,10,104).

Several evidence suggested that the source of fungi and bacteria in indoor air are influenced by many factors, such as human occupancy and their activities, humidity levels, ventilation, environmental characteristics, water infiltrations, construction and decoration materials and outdoor air (9,34,77). Concerning *SARS-CoV-2* virus, it is transmitted by aerosols and through contaminated objects and surfaces, including human skin (16,51,56,57,105). Other factors can influence the infection, such as temperature, humidity and ventilation and filtering system (16). Due to the extensive list of factors that influence IAQ, exposure assessment to microorganisms remains a challenge to every exposure assessor/industrial hygienist (74). The sampling approach is of critical importance to achieve an accurate risk characterization regarding microbiological agents (106). Active methods (air sampling), such as impingement method used in this study, although the most used method for samples collection, may not represent the real scenario regarding the inhalation exposure (46,77,107). Besides that, can only reflect the load from a shorter period of time (mostly minutes), corresponding to the sampling duration and representing only a small fraction of the bioburden exposure (46,77,107). Despite these concerns, impingers are among the most common sampling devices used for *SARS-Cov-2* assessment in indoor environments, since it allows longer active sampling times to ensure collection of sufficient airborne viruses for detection by molecular tools (51,57,108). On the other hand, passive methods, such as surface swabs used in this study, allow the collection of contamination over a longer period (after the last cleaning procedure) and to observe a wider diversity of the bioburden, providing a more complete picture of the exposure than the active sampling (46,77,106,107). Indeed, swabs are also frequently used in

several indoor environments, including for *SARS-CoV-2* (24,51,57,72,101,106,109–116). The use of both sampling methods allows to overcome each method limitation, ensuring a more precise assessment of occupational exposure to bioburden (51,77). Although most of the studies performed in HEI are focused on the air (18,59,61,63,68,70), surface analysis has been shown to be relevant, because it may reflect the contamination in the air, thus possibly leading to increase in airborne concentration (21,24,117). It allows also the measurement of the harmful part of the airborne population which falls onto a critical surface (74,75,78).

Previous studies have shown that, beyond sampling approach, culture media applied also influence the results obtained for fungi and bacteria found in environments (101,106). Regarding bacterial contamination, culture media allowed for the discrimination between total bacteria (TSA media) and Gram-negative bacteria (VRBA media) (101). The presence of bacteria on surfaces is a common situation, especially in the most frequently touched surfaces, as their main source is people and their activities (41,42,48). Tacking this into account, it is not surprising that the highest total bacterial count was found in the offices (31.8%), followed by auditorium (15.7%) and meals space (14.4%), which are the places with high occupancy and where there are several touched surfaces for different workers. In fact, meals space is a place that involves the handling of raw material (vegetables, fruits, meats, and others) that have bacteria content (7). Incorrect hygiene practices of workers must be considered since the bacteria are mainly from humans (7). Thus, the identification of bacterial species present in this area it is an important tool, for conclude its origin (7). The positive correlation found between higher bacterial counts in TSA and higher number of workers emphasizes this contribution.

On the other hand, Gram-negative bacteria presented the highest levels on laboratories (26.8%), workshops (25.4%), and offices (15.5%). This can be a result of the activities that are carried out in these areas, especially in laboratories, which are spaces where people can deal with a complex mixture of microorganisms, chemicals, and contaminants due to the nature of research (18,60). The deposition of these bacteria on surfaces together with inadequate hygienic practices can lead to their presence on other surfaces and transferred to people, which can be harmful to occupants (62). Moreover, previous studies have linked the presence of Gram-negative bacteria to the settled dust brought into indoor environment by users and from outdoor particles (109).

HEI 7 and HEI 3 have a higher amount of total bacteria and Gram-negative bacteria, respectively. The sampled areas can be a justification for these results, since both included the same three more prevalent spaces for all institutes, with the exception for HEI 3, where no samples were collected in offices.

Besides bacteria are ubiquitous and generally of human origin (from skin and mucous membranes) and not harmful for health, the mere presence of Gram-negative bacteria is a special concern, as they may have naturally resistance to antibiotics and can also produce endotoxins, which can cause respiratory symptoms (9,24,34,42).

Aiming the fungal contamination assessment, besides the use of MEA, as suggested in Portuguese guidance for IAQ assessment (9), DG18 was also selected to be used, since this media constitutes a better alternative for colony counting and to obtain higher diversity of genera, since contains dichloran, which is an anti-fungal agent that inhibits bacterial growth and restrict the spread of more-rapidly growing fungi, such as fungi belonging to the Mucorales order (including *Rhizopus* and *Mucor* genera) and restricts the colony size of other genera (46,118). However, although the total concentrations obtained in both media are similar, contrary to expectations, a greater diversity of the mycobiota was found in MEA (10 different species) than in DG18 (9 different species). The same trend was observed in relation to *Aspergillus* sections (5 in MEA; 3 in DG18). The fact that was identified different species in MEA and in DG18 emphasizes the use of both media.

In the present study the concentration of fungi on the surfaces was lower than the concentration of bacteria, which is similar to a study on surface swabs in university facilities (7). The most prevalent fungi found on this study, such as *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp. were in accordance with others where the surfaces swabs were also used as sampling approach (21,89,119,120).

Like bacteria, fungi are present in dust and surfaces of every house (34). However, previous studies suggested that fungi originate mainly from outdoors and can be transferred to the building on the surface of new materials or by humans (34,36,37,121). Ventilation and daily activities can also promote mold indoors (34,36).

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 were professor's room (32.0%), offices (24.0%) and changing and dressing room (11.8%). In both media, it is possible to observe fungal contamination in offices, which resembles bacteria. A possible reason for the presence of fungi in these areas could be the fact that, due to the COVID-19 pandemic, all indoor spaces were encouraged to open windows to

prevent COVID-19 infection (16). Evidences concluded that opening the windows appears to increase the levels of associated microorganisms in the air and on the surfaces as a result of the passage of outdoor air into indoors (18,48,104,121). In addition, it cannot be ruled out that people can touch surfaces and transferred fungi ant their activities can promote their dissemination (5,36). This is particularly interesting for changing and dressing room, where the materials involved (clothes, skin, hair and shoes) can be a human transmission route (34,36). Fungal growth can also 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 (34,36,48).

HEI 6 and HEI 2 have highest values of total fungal contamination on MEA and DG18 media, respectively. As seen in bacteria, the areas sampled in HEI 6 showed the same three most prevalent spaces for all institutes on MEA. In HEI 2 on DG18, among the areas with higher contamination values in all institutes, only professor's rooms were analyzed. However, in this mentioned area a considerable concentration of fungi was evident.

Besides fungi are present in all buildings, some of the species can cause negative health impacts, such as respiratory conditions, inflammatory, allergic and toxin effects (36,38,39). Its ability to produce mycotoxins, which can have adverse health effects, including death in the worst cases, is of special concern and should be studied in more detailed (40–42).

Aspergillus sections has been object of study as most of them are reported as mycotoxins producers, some with clinical relevance (47). While in MEA was exclusively identified *Aspergillus* sections *Fumigati*, *Nigri* and *Aspergilli*, *Aspergillus* section *Circumdati* was isolated only on DG18 media (as previously reported (106)). These *Aspergillus* sections are indicators of harmful fungal contamination (39,84,122). Furthermore, the presence of *Aspergillus* sections *Candidi* and *Nidulantes in* both media should be emphasized due to their toxigenic potential (46,84).

The complementarity of the results reflects the importance of using both media to achieve a more accurate mycobiota exposure assessment (89).

The emergence of pathogenic fungi resistant to the antifungal agents has been notorious in recent years, which can cause therapeutic failure (43,44). In this study, the screening of fungal resistance to three medical azoles, widely used in the treatment of fungal infections, was conducted (43,44). *Cladosporium* sp. was the most identified fungal species in all three azoles (64.71% on ITR; 65.49% on VOR; 53.33% on POS).

Penicillium sp. also growth in all three azoles being the second species more prevalent in two of the three azoles (14.79% on VOR; 22.22% on POS). *Chrysosporium* sp., *Chrysonilia sitophila* and *Mucor* sp. were presented also 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. Regarding *Mucor* sp. and *Rhizopus* sp., they are intrinsically resistant to VOR, with ITR and POS as first-line therapy (77,123). (77,123). However, in this study, there were observed the growth of these fungal species in the presence of ITR and POS thus indicating the need to further characterize fungal resistance of those isolates (47,123).

The identification of fungal species in in more than one azole suggests a multi drug resistance phenotype that must be further evaluated through antifungal susceptibility testing (123), by the reference microdilution method (EUCAST) (124). 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 (24). However, no conclusions regarding azole resistance of these fungal species can be drawn, as reference values are defined only for *Aspergillus* sp. and *Candida* sp. (126).

Antifungal resistance is emerging, especially in *Aspergillus fumigatus*, which is the main cause of invasive fungal infections (39,125). In this study, only *Aspergillus* section *Nigri* was identified in one azole media (0.70% on VOR). Nevertheless, *Aspergillus* sections *Candidi*, *Fumigati*, *Nidulantes*, *Nigri*, and *Circumdati* were observed in control SAB. Thus, some of those sections were found also in MEA and/or DG18. These results are in line with some studies in dwellings and hospitals environments, where no *Aspergillus* species were able to grow on azole-media, despite being observed in SAB, MEA or DG18 (24,77,101,114). 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 (24,77,101).

Culture-based methods allowed the identification of the *Aspergilllus* section *Fumigati* in a wide number of surface samples, with molecular tools also detecting this section in different and in a smaller number of samples. Despite this discrepancy, the use of both methods allows to obtain a broader picture of fungal contamination, as supported by other authors (37,39,80,89).

In fact, although molecular technique has some drawbacks, specific characteristics allow them to be more successful than other techniques, such as its precision, speed, intense analytical sensitivity of detection and the fact that it enables the detection and

identification of dead or dormant microorganisms and can discriminate toxigenic strains of some fungal species (86,126). On the other hand, culture-based 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 which reinforces the idea of combining both methods (123).

In all air samples, was not possible to detect *Aspergillus* section *Fumigati*. A recent review article concluded that, although in most studies the number of molecular detections of *Fumigati* section was higher than the number of positive identifications in culture medium, there were cases where detection by qPCR was not possible (47). For example, a study performed in an animal feed industry detected only one of the samples in the presence of *Aspergillus* section *Fumigati* by molecular methods, in contrast to the high prevalence of this section presented by culture-based methods (127). Interestingly, this specie was not at high levels in this sample when detected by culture-based methods(127). The lack of correlation between both methods has been reported by other authors and it can be seen that qPCR possible may yield false negatives (47,77,86,127). This could be a result of inadequate removal for PCR inhibitors in the samples, ineffective release of microbial DNA content from cells or poor DNA recovery after extraction and purification steps (47,77,86,127). This study is in line with another that pointed out the presence of particle in the air as a possible reason for sources of inhibition (127). To ensure the results on air samples, the use of culture-based methods should be performed. Once again the importance of complementing the use of both methods is evident (37,39,80,89).

Regarding *SARS-CoV-2* results, all samples collected were negative after molecular detection by RT-qPCR. This can indicate an effective cleaning and a good ventilation of the areas, as recommended by experts, include Portuguese Health Directorate (16,57). The fact that the results were negative in both sampling methods shows their complementarity and allows corroborating the results. This assessment has a great impact on the safety of people using the HEI under study, since the implementation of measures seems to be prevented the spread *of SARS-CoV-2* in the air and surface. However, regular detection of *SARS-CoV-2* in environmental samples should be maintained.

6. Final considerations

6.1. Conclusions

The present study was important due to the lack of research in IAQ on HEI, including the assessment of microbiological contamination. The high relevance of this study relies on the great diversity of occupants, who can spend a lot of time in the facilities. Additionally, the presence of microorganisms can cause negative health effects and affect the productivity and performance of workers and students. Besides that, the presence of *SARS-CoV-2* is a public health concern, and its assessment in these settings allows ensure the safety of all occupants. Some innovative aspects can be highlighted, such as the fact that the azole resistance screening was performed, something normally carried out only in clinical settings. It can also be emphasized the analysis of fungi and bacteria on surfaces, which is sometimes neglected. It is important to point out that a technical-scientific report was prepared for the person responsible for HEI (See Appendix 8.2 – Scientific technical report). This report established recommendations to reduce the microbiological contamination found.

This study showed that although cleaning and disinfection procedures are done regularly due to the COVID-19 pandemic, surfaces were often contaminated with fungi and bacteria. This can be a result of increasing resistance to biocides and the wide range of environmental factors that can contribute to the dissemination of microbial contamination indoors. Furthermore, the growth of fungal species in azoles should be considered.

However, it is important to mention that in the case of *SARS-CoV-2* its presence was not detected, which can be related to an effective cleaning and disinfection to eliminate the virus. In addition, although on surface samples was detected *Aspergillus* sp., including section *Fumigati*, which has clinical relevance, on air samples this section was not identified by qPCR.

The use of surface swabs proved to be a valuable sampling method for the assessment of microbiological contamination. Thus, active and passive sampling methods used in parallel with culture-based methods and molecular tools showed a more precise characterization of the contamination. Therefore, corrective measures should be implemented to reduce bacterial and fungal presence.

6.2. Study limitations

A limitation of this study is the fact that the active sampling method (air impingement), except for the molecular identification of *Aspergillus* section *Fumigati* by molecular tool, was not applied to determine fungal and bacterial contamination, which could complement both results (74,77,106).

Since seasonality influences the concentration of microorganisms, the fact that sampling was performed between summer and autumn may constitute a limitation (1,63).

Furthermore, no antifungal susceptibility testing was performed on isolates that growth on azoles and that can cause human infections (24,47).

6.3. Suggestions for future studies

As suggestion for future studies, additional research to correlated air and surface can be interesting to find the source of contamination. Considering the variations in the levels of microbiological contamination over time, it is recommended that in the future a more extensive study be carried out throughout the year, covering all seasons. As some *Aspergillus* sections detected in this study can produce mycotoxins, it would be important to analyze their presence in these samples. Fungal species that grew on azoles would be interesting to study in the future to characterize the fungal resistance of these isolates. Taking into account the levels of bacterial and fungal contamination found, further similar studies should be carried out on a regular basis, including the detection of *SARS-CoV-2*.

6.4. Ethical and Legal considerations

In order to ensure anonymity and confidentiality, the name of the HEI under study were not available. With regard to legal considerations, all institutions involved authorize the work to be carried out.

7. References

1. Di Giulio M, Grande R, Di Campli E, Di Bartolomeo S, Cellini L. Indoor air quality in university environments. Environ Monit Assess [Internet]. 2010;170(1–4):509–17. Available from: https://link.springer.com/article/10.1007/s10661-009-1252-7
2. Hayleeyesus SF, Manaye AM. Microbiological quality of indoor air in University libraries. Asian Pac J Trop Biomed [Internet]. 2014;4(4):S312–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4025286/
3. Cincinelli A, Martellini T. Indoor air quality and health. Int J Environ Res Public Health [Internet]. 2017;14(11). Available from: https://www.mdpi.com/1660-4601/14/11/1286
4. M. Stryjakowska-Sekulska, A. Piotraszewska-Pająk, A. Szyszka MN, Filipiak M. Microbiological quality of indoor air in University Rooms. Polish J Env [Internet]. 2007;16(4):623–32. Available from: http://www.pjoes.com/Microbiological-Quality-of-Indoor-Air-r-nin-University-Rooms,88030,0,2.html
5. Ross AA, Neufeld JD. Microbial biogeography of a university campus. Microbiome [Internet]. 2015;3:66. Available from: https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-015-0135-0
6. Erlandson G, Magzamen S, Carter E, Sharp JL, Reynolds SJ, Schaeffer JW. Characterization of indoor air quality on a college campus: A pilot study. Int J Environ Res Public Health [Internet]. 2019;16(15):17–26. Available from: https://www.mdpi.com/1660-4601/16/15/2721
7. Haleem AM, Hassan DMA, Al-hiyaly SAK. Comparative Assessment of Microbial Contamination from Swabs collected within University Facilities. J Heal Sci [Internet]. 2013;3(2). Available from: http://article.sapub.org/10.5923.j.health.20130302.04.html
8. United States Environmental Protection Agency. Introduction to Indoor Air Quality [Internet]. [cited 2021 Apr 24]. Available from: https://www.epa.gov/indoor-air-quality-iaq/introduction-indoor-air-quality
9. Agência Portuguesa do Ambiente. Qualidade do Ar em Espaços Interiores Um Guia Técnico 2010. 2010;
10. Tran V Van, Park D, Lee YC. Indoor Air Pollution, Related Human Diseases, and Recent Trends in the Control and Improvement of Indoor Air Quality. Int J Environ Res Public Health [Internet]. 2020;17(8). Available from:

https://www.mdpi.com/1660-4601/17/8/2927

11. Duarte N, Roa S, Méndez-C F. Microbiological Air Quality in an Optical Clinic. Cienc y Tecnol para la Salud Vis y Ocul [Internet]. 2019;17(1):19–28. Available from: https://ciencia.lasalle.edu.co/svo/vol17/iss1/2/
12. Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: Progress and prospects. Ann Occup Hyg [Internet]. 2003;47(3):187–200. Available from: https://academic.oup.com/annweh/article/47/3/187/171690
13. Moelling K, Broecker F. Air Microbiome and Pollution: Composition and Potential Effects on Human Health, including SARS Coronavirus Infection. J Environ Public Health [Internet]. 2020;2020. Available from: https://www.hindawi.com/journals/jeph/2020/1646943/
14. Ijaz MK, Zargar B, Wright KE, Rubino JR, Sattar SA. Generic aspects of the airborne spread of human pathogens indoors and emerging air decontamination technologies. Am J Infect Control [Internet]. 2016;44(9):S109–20. Available from: https://www.sciencedirect.com/science/article/pii/S0196655316305363
15. World Health Organisation. Antimicrobial resistance [Internet]. 2020 [cited 2021 Feb 5]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance
16. 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 [Internet]. 2020;25(1):1–17. Available from: https://environhealthprevmed.biomedcentral.com/articles/10.1186/s12199-020-00904-2
17. United States Environmental Protection Agency. How Does Indoor Air Quality Impact Student Health and Academic Performance? [Internet]. [cited 2021 Jun 24]. Available from: https://www.epa.gov/iaq-schools/how-does-indoor-air-quality-impact-student-health-and-academic-performance
18. Jurado SR, Bankoff ADP, Sanchez A. Indoor air quality in Brazilian universities. Int J Environ Res Public Health [Internet]. 2014;11(7):7081–93. Available from: https://www.mdpi.com/1660-4601/11/7/7081
19. Madureira J, Aguiar L, Pereira C, Mendes A, Querido MM, Neves P, et al. Indoor exposure to bioaerosol particles: levels and implications for inhalation dose rates in schoolchildren. Air Qual Atmos Heal [Internet]. 2018;11(8):955–64. Available

from: https://link.springer.com/article/10.1007/s11869-018-0599-8

20. Górny RL. Microbial aerosols: Sources, properties, health effects, exposure assessment—A review. KONA Powder Part J [Internet]. 2020;37(37):64–84. Available from: https://www.jstage.jst.go.jp/article/kona/37/0/37_2020005/_html/-char/en
21. Viegas C, Faria T, Meneses M, Carolino E, Viegas S, Gomes AQ, et al. Analysis of surfaces for characterization of fungal burden - Does it matter? Int J Occup Med Environ Health [Internet]. 2016;29(4):623–32. Available from: http://ijomeh.eu/Analysis-of-surfaces-for-characterization-of-fungal-burden-Does-it-matter-,59242,0,2.html
22. The Government of the Hong Kong Special Administrative Region Indoor Air Quality Management. Guidance Notes for the Management of Indoor Air Quality in Offices and Public Places. 2003.
23. Sundell J. On the history of indoor air quality and health. Indoor Air, Suppl [Internet]. 2004;14(SUPPL. 7):51–8. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1600-0668.2004.00273.x
24. Viegas C, Almeida B, Monteiro A, Caetano LA, Carolino E, Gomes AQ, et al. Bioburden in health care centers: Is the compliance with Portuguese legislation enough to prevent and control infection? Build Environ [Internet]. 2019;160(106226). Available from: https://www.sciencedirect.com/science/article/pii/S0360132319304366
25. 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 [Internet]. 2019;28(10):1307–23. Available from: https://journals.sagepub.com/doi/abs/10.1177/1420326X19833118
26. Hwang SH, Roh J, Park WM. Evaluation of PM10, CO2, airborne bacteria, TVOCs, and formaldehyde in facilities for susceptible populations in South Korea. Environ Pollut [Internet]. 2018;242:700–8. Available from: https://www.sciencedirect.com/science/article/pii/S0269749118314386?via%3Dihub
27. Brągoszewska E, Biedroń I, Kozielska B, Pastuszka JS. Microbiological indoor air quality in an office building in Gliwice, Poland: analysis of the case study. Air Qual Atmos Heal [Internet]. 2018;11(6):729–40. Available from: https://link.springer.com/article/10.1007/s11869-018-0579-z
28. Guo H, Lee SC, Chan LY. Indoor air quality investigation at air-conditioned and

non-air-conditioned markets in Hong Kong. Sci Total Environ [Internet]. 2004;323(1–3):87–98. Available from: https://www.sciencedirect.com/science/article/pii/S0048969703005825

29. Majd E, McCormack M, Davis M, Curriero F, Berman J, Connolly F, et al. Indoor air quality in inner-city schools and its associations with building characteristics and environmental factors. Environ Res [Internet]. 2019;170:83–91. Available from: https://www.sciencedirect.com/science/article/pii/S0013935118306406
30. 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.
31. Śmiełowska M, Marć M, Zabiegała B. Indoor air quality in public utility environments—a review. Environ Sci Pollut Res [Internet]. 2017;24(12):11166–76. Available from: https://link.springer.com/article/10.1007/s11356-017-8567-7
32. United States Environmental Protection Agency. Biological Pollutants' Impact on Indoor Air Quality [Internet]. United States Environmental Protection Agency. 2018 [cited 2021 Feb 3]. Available from: https://www.epa.gov/indoor-air-quality-iaq/biological-pollutants-impact-indoor-air-quality
33. World Health Organisation. Development of WHO Guidelines for Indoor Air Quality - Report on a Working Group Meeting. World Health Organization Regional Office for Europe. 2006.
34. World Health Organisation. WHO guidelines for indoor air quality: dampness and mould. World Heal Organ Reg Off Eur. 2009;
35. The Goverment of the Hong Kong Special Administrative Region. Indoor Air Quality Management Group. Guidance Notes for the Management of Indoor Air Quality in Offices and Public Places. 2019;
36. Nevalainen A, Täubel M, Hyvärinen A. Indoor fungi: Companions and contaminants. Indoor Air [Internet]. 2015;25(2):125–56. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/ina.12182
37. Cox J, Mbareche H, Lindsley WG, Duchaine C. Field sampling of indoor bioaerosols. Aerosol Sci Technol [Internet]. 2020;54(5):572–84. Available from: https://www.tandfonline.com/doi/full/10.1080/02786826.2019.1688759
38. Tong X, Xu H, Zou L, Cai M, Xu X, Zhao Z, et al. High diversity of airborne fungi in the hospital environment as revealed by meta-sequencing-based microbiome analysis. Sci Rep [Internet]. 2017;7(39606). Available from: https://www.nature.com/articles/srep39606

39. Sabino R, Veríssimo C, Viegas C, Viegas S, Brandão J, Alves-Correia M, et al. The role of occupational Aspergillus exposure in the development of diseases. Med Mycol [Internet]. 2019;57:S196–205. Available from: https://academic.oup.com/mmy/article/57/Supplement_2/S196/5366897
40. Sharma D, Dutta BK, Singh AB. Exposure to indoor fungi in different working environments: A comparative study. Aerobiologia (Bologna) [Internet]. 2010;26(4):327–37. Available from: https://link.springer.com/article/10.1007/s10453-010-9168-9
41. Kim KH, Kabir E, Jahan SA. Airborne bioaerosols and their impact on human health. J Environ Sci (China) [Internet]. 2018;67:23–35. Available from: https://www.sciencedirect.com/science/article/pii/S100107421731478X
42. Moldoveanu AM. Biological Contamination of Air in Indoor Spaces. In: Farhad Nejadkoorki, editor. Current Air Quality Issues. 2015.
43. Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect Dis [Internet]. 2017;17(12):e383–92. Available from: https://www.sciencedirect.com/science/article/pii/S147330991730316X
44. Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science (80-) [Internet]. 2018;360(6390):739–42. Available from: https://science.sciencemag.org/content/360/6390/739
45. Nature microbiology. Stop neglecting fungi [Internet]. Vol. 2, Nature microbiology. 2017. Available from: https://www.nature.com/articles/nmicrobiol2017120
46. 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 (Basel) [Internet]. 2021;12(1):1–12. Available from: https://www.mdpi.com/2073-4433/12/1/23
47. Viegas C, Caetano LA, Viegas S. Occupational exposure to Aspergillus section Fumigati: Tackling the knowledge gap in Portugal. Environ Res [Internet]. 2021;194(110674). Available from: https://www.sciencedirect.com/science/article/pii/S0013935120315711
48. Nazaroff WW. Indoor bioaerosol dynamics. Indoor Air [Internet]. 2016;26(1):61–78. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/ina.12174
49. Smets W, Moretti S, Denys S, Lebeer S. Airborne bacteria in the atmosphere:

Presence, purpose, and potential. Atmos Environ [Internet]. 2016;139(2016):214–21. Available from: https://www.sciencedirect.com/science/article/pii/S1352231016303855

50. McEwen SA, Collignon PJ. Antimicrobial Resistance: a One Health Perspective. Antimicrob Resist Bact from Livest Companion Anim [Internet]. 2018;521–47. Available from: https://journals.asm.org/doi/10.1128/microbiolspec.ARBA-0009-2017
51. Pena P, Morais J, Quintal A, Viegas C. Sampling methods and assays applied in SARS-CoV-2 exposure assessment. Sci Total Environ [Internet]. 2021;775:145903. Available from: https://www.sciencedirect.com/science/article/pii/S0048969721009700?via%3Dihub
52. World Health Organisation. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 [Internet]. 2020 [cited 2021 May 3]. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020
53. Astuti I, Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes Metab Syndr Clin Res Rev. 2020;14(4):407–12.
54. Cevik M, Kuppalli K, Kindrachuk J, Peiris M. Virology, transmission, and pathogenesis of SARS-CoV-2. BMJ [Internet]. 2020;371. Available from: https://www.bmj.com/content/371/bmj.m3862
55. World Health Organisation. WHO Coronavirus (COVID-19) Dashboard [Internet]. [cited 2021 Jul 19]. Available from: https://covid19.who.int/
56. Dargahi A, Jeddi F, Vosoughi M, Karami C, Hadisi A, Ahamad Mokhtari S, et al. Investigation of SARS CoV-2 virus in environmental surface. Environ Res [Internet]. 2021;195(October 2020):110765. Available from: https://www.sciencedirect.com/science/article/pii/S0013935121000591
57. Borges JT, Nakada LYK, Maniero MG, Guimarães JR. SARS-CoV-2: a systematic review of indoor air sampling for virus detection. Environ Sci Pollut Res [Internet]. 2021;2019. Available from: https://link.springer.com/article/10.1007/s11356-021-13001-w
58. Xiuqin L, Zhiguo F, Chanjuan G. Assessment of culturable airborne fungi in a university campus in Hangzhou, southeast China. African J Microbiol Res

[Internet]. 2012;6(6):1197–205. Available from: https://academicjournals.org/journal/AJMR/article-abstract/4CC373713532

59. Asif A, Zeeshan M, Jahanzaib M. Assessment of indoor and outdoor microbial air quality of cafeterias of an educational institute. Atmos Pollut Res [Internet]. 2019;10(2):531–6. Available from: https://www.sciencedirect.com/science/article/pii/S1309104218304045
60. Idris SA 'Ainaa,' Hanafiah MM, Ismail M, Abdullah S, Khan MF. Laboratory air quality and microbiological contamination in a university building. Arab J Geosci [Internet]. 2020;13(13):580. Available from: https://link.springer.com/article/10.1007%2Fs12517-020-05564-8
61. Onet A, Ilies DC, Buhas S, Rahota D, Ilies A, Baias S, et al. Microbial air contamination in indoor environment of university sports hall. J Environ Prot Ecol. 2018;19(2):694–703.
62. Zulkafar SS, Abu Hassan MF, Abu Bakar NF. Microbiological Assessment of Selected Laboratories at a Local University in Malaysia. J Sains Kesihat Malaysia. 2019;17(SI):119–26.
63. Kic P, Růžek L. The microbiological environment in specific rooms of a university campus. Agron Res [Internet]. 2014;12(3):837–42. Available from: https://agronomy.emu.ee/vol123/2014_3_18_b5.pdf
64. 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 (Bologna) [Internet]. 2020;36(3):313–24. Available from: https://link.springer.com/article/10.1007%2Fs10453-020-09633-z
65. Crowe J, Schnaubelt AT, Scott S-B, Kathleen A, Bai J, Eske T, et al. Pilot program for test-based SARS-CoV-2 screening and environmental monitoring in an urban public school district. 2021; Available from: https://www.medrxiv.org/content/10.1101/2021.04.14.21255036v1
66. Chan VWM, So SYC, Chen JHK, Yip CCY, Chan KH, Chu H, et al. Air and environmental sampling for SARS-CoV-2 around hospitalized patients with coronavirus disease 2019 (COVID-19). Infect Control Hosp Epidemiol [Internet]. 2020;41(11):1258–65. Available from: https://www.cambridge.org/core/journals/infection-control-and-hospital-epidemiology/article/air-and-environmental-sampling-for-sarscov2-around-hospitalized-patients-with-coronavirus-disease-2019-

covid19/2603FF55AA16BB839ED5A500DF62EFAE

67. Li Y, Ge Y, Liu J, Wang F, Lv H, Jin M. Investigation and evaluation of bacterial contaminant in classrooms and dormitories of college students in winter: A study in a university of Tianjin, China. Indoor Air 2014 - 13th Int Conf Indoor Air Qual Clim [Internet]. 2014;717–21. Available from: https://www.researchgate.net/publication/289013481_Investigation_and_evaluation_of_bacterial_contaminant_in_classrooms_and_dormitories_of_college_students_in_winter_A_study_in_a_university_of_Tianjin_China
68. 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 [Internet]. 2017;0(0):1–12. Available from: https://journals.sagepub.com/doi/10.1177/1420326X17748463?icid=int.sj-abstract.similar-articles.2
69. Landry KG, Ascension NM, Armelle DCI, Hortense GK, François-Xavier E. Assessment of Indoor Microbial Quality of Library's Premise: Case of Central Library of the University of Yaoundé I. Open J Prev Med [Internet]. 2018;08(04):109–20. Available from: https://www.scirp.org/journal/paperinformation.aspx?paperid=83985
70. Grisoli P, Rodolfi M, Chiara T, Zonta LA, Dacarro C. Evaluation of microbiological air quality and of microclimate in university classrooms. Environ Monit Assess [Internet]. 2012;184(7):4171–80. Available from: https://link.springer.com/article/10.1007/s10661-011-2253-x
71. Guo K, Qian H, Zhao D, Ye J, Zhang Y, Kan H, et al. Indoor exposure levels of bacteria and fungi in residences, schools, and offices in China: A systematic review. Indoor Air [Internet]. 2020;30(6):1147–65. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ina.12734
72. Cabo Verde S, Almeida SM, Matos J, Guerreiro D, Meneses M, Faria T, et al. Microbiological assessment of indoor air quality at different hospital sites. Res Microbiol [Internet]. 2015;166(7):557–63. Available from: https://www.sciencedirect.com/science/article/pii/S092325081500056X?via%3Dihub
73. Diário da República 1.ª série - Nº 126. Saúde e ambiente e ação climática. Portaria n.º 138-G/2021 [Internet]. 2021. Available from: https://dre.pt/web/guest/home/-/dre/166296490/details/maximized

74. Viegas C. Sampling methods for an accurate mycobiota occupational exposure assessment—overview of several ongoing projects [Internet]. London: CR. Al OS and HVA et, editor. Occupational Safety and Hygiene VI - Selected contributions from the International Symposium Occupational Safety and Hygiene, SHO 2018. 2018. 7–11 p. Available from: https://www.researchgate.net/publication/324043578_Sampling_methods_for_an_accurate_mycobiota_occupational_exposure_assessment_-_Overview_of_several_ongoing_projects
75. Mousavi MS, Hadei M, Majlesi M, Hopke PK, Yarahmadi M, Emam B, et al. Investigating the effect of several factors on concentrations of bioaerosols in a well-ventilated hospital environment. Environ Monit Assess [Internet]. 2019;191(7):407. Available from: https://link.springer.com/article/10.1007/s10661-019-7559-0
76. Napoli C, Marcotrigiano V, Montagna MT. Air sampling procedures to evaluate microbial contamination: A comparison between active and passive methods in operating theatres. BMC Public Health [Internet]. 2012;12(594). Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-12-594
77. Viegas C, Almeida B, Monteiro A, Paciência I, Rufo JC, Carolino E, et al. Settled dust assessment in clinical environment: useful for the evaluation of a wider bioburden spectrum. Int J Environ Health Res [Internet]. 2021;31(2):160–78. Available from: https://www.tandfonline.com/doi/full/10.1080/09603123.2019.1634799
78. Setlhare G, Malebo N, Shale K, Lues R. Identification of airborne microbiota in selected areas in a health-care setting in South Africa. BMC Microbiol [Internet]. 2014;14(100). Available from: https://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-14-100
79. Stockwell RE, Ballard EL, O'Rourke P, Knibbs LD, Morawska L, Bell SC. Indoor hospital air and the impact of ventilation on bioaerosols: a systematic review. J Hosp Infect [Internet]. 2019;103(2):175–84. Available from: https://www.sciencedirect.com/science/article/pii/S0195670119302786
80. Yoo K, Lee TK, Choi EJ, Yang J, Shukla SK, Hwang S il, et al. Molecular approaches for the detection and monitoring of microbial communities in bioaerosols: A review. J Environ Sci (China) [Internet]. 2017;51:234–47. Available from: https://www.sciencedirect.com/science/article/pii/S1001074216302418?via%3Di

hub

81. Zemouri C, De Soet H, Crielaard W, Laheij A. A scoping review on bio-Aerosols in healthcare & the dental environment. PLoS One [Internet]. 2017;12(5):1–26. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0178007
82. Eduard W, Heederik D. Methods for quantitative assessment of airbone levels of noinfectious microorganisms in highly contaminated work environment. Am Ind Hyg Assoc J [Internet]. 1998;59(2):113–27. Available from: https://www.tandfonline.com/doi/abs/10.1080/15428119891010370
83. Figdor D, Gulabivala K. Survival against the odds: microbiology of root canals associated with post-treatment disease. Endod Top [Internet]. 2011;18(1):62–77. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1601-1546.2011.00259.x
84. Viegas C, Faria T, Caetano LA, Carolino E, Gomes AQ, Viegas S. Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings? J Occup Environ Hyg [Internet]. 2017;14(10):771–85. Available from: https://www.tandfonline.com/doi/full/10.1080/15459624.2017.1334901
85. Degois J, Clerc F, Simon X, Bontemps C, Leblond P, Duquenne P. First metagenomic survey of the microbial diversity in bioaerosols emitted in waste sorting plants. Ann Work Expo Heal [Internet]. 2017;61(9):1076–86. Available from: https://academic.oup.com/annweh/article/61/9/1076/4210020
86. Viegas C, Almeida B, Gomes AQ, Carolino E, Caetano LA. Aspergillus spp. prevalence in Primary Health Care Centres: Assessment by a novel multi-approach sampling protocol. Environ Res [Internet]. 2019;175:133–41. Available from: https://www.sciencedirect.com/science/article/pii/S0013935119302737
87. McDevitt JJ, Lees PSJ, Merz WG, Schwab KJ. Inhibition of quantitative PCR analysis of fungal conidia associated with indoor air particulate matter. Aerobiologia (Bologna) [Internet]. 2007;23(1):35–45. Available from: https://link.springer.com/article/10.1007/s10453-006-9047-6
88. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: Uses, limitations, and future applications in acute-care settings. Lancet Infect Dis [Internet]. 2004;4(6):337–48. Available from: https://www.sciencedirect.com/science/article/pii/S1473309904010448
89. Viegas C, Faria T, Monteiro A, Caetano LA, Carolino E, Gomes AQ, et al. A

novel multi-approach protocol for the characterization of occupational exposure to organic dust-Swine production case study. Toxics [Internet]. 2018;6(1):1–14. Available from: https://www.mdpi.com/2305-6304/6/1/5

90. Viegas C, Dias M, Gomes B, Pimenta, R, Oliveira K, Delgadinho M et al. Towards and integrated approach on Occupational Health to tackle COVID19 pandemic. Submitt Publ. 2021;
91. Viegas C, Pimenta R, Dias M, Gomes B, Aranha Caetano L, Brito M et al. Microbiological contamination assessment in higher education institutes. Submitt Publ. 2021;
92. Bertin Technologies SAS. CORIOLIS MICRO – AIR SAMPLER [Internet]. [cited 2021 Apr 19]. Available from: https://www.bertin-instruments.com/product/air-samplers/coriolis-micro-air-sampler/
93. Morris G, Kokki MH, Anderson K, Richardson MD. Sampling of Aspergillus spores in air. J Hosp Infect [Internet]. 2000;44(2):81–92. Available from: https://www.sciencedirect.com/science/article/pii/S0195670199906886
94. Nzytech. NZY Viral RNA Isolation kit [Internet]. [cited 2021 Apr 7]. Available from: https://www.nzytech.com/products-services/molecular-biology/dnarna-purification/rna-purification-dnarna-purification/mb40701/
95. American National Standards Institute (ANSI). ISO 18593: 2004 - Microbiology of Food and Animal Feeding Stuffs, Horizontal Methods for Sampling Techniques from Surfaces Using Contact Plates and Swabs.
96. United States Environmental Protection Agency. EPA Observational Economy Series Volume 1: Composite Sampling. 1995;1(August). Available from: https://www.epa.gov/sites/production/files/2016-03/documents/comp-samp.pdf
97. Direção-Geral da Saúde. Estudo define as linhas vermelhas para intervenção na epidemia por COVID-19 [Internet]. 2021 [cited 2021 Jul 28]. Available from: https://covid19.min-saude.pt/estudo-define-as-linhas-vermelhas-para-intervencao-na-epidemia-por-covid-19/
98. The University of Adelaide. Mycology Home. Fungal Descriptions and Antifungal Susceptibility. Hyphomycetes [Internet]. Mycology Online. [cited 2021 Jul 28]. Available from: https://mycology.adelaide.edu.au/descriptions/hyphomycetes/aspergillus/
99. European Committee on Antimicrobial Susceptibility Testing. Antifungal Breakpoint Tables for Interpretation of MICs v9.0 3–7. 2018.
100. De Hoog C, Guarro J, Gene G, Figueras M. Atlas of Clinical Fungi – The

ultimate benchtool for diagnosis. Version 4.1.4. Utrecht: Centraalbureau voor Schimmelcultures. Utrecht: Centraalbureau voor Schimmelcultures, Utrecht; 2016.

101. Viegas C, Twaruzek M, Lourenço R, Dias M, Almeida B, Caetano LA, 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 (Basel) [Internet]. 2020;11(4):351. Available from: https://www.mdpi.com/2073-4433/11/4/351/htm
102. Cruz-Perez P, Buttner MP, Stetzenbach LD. Detection and quantitation of Aspergillus fumigatus in pure culture using polymerase chain reaction. Mol Cell Probes [Internet]. 2001;15(2):81–8. Available from: https://www.sciencedirect.com/science/article/pii/S0890850800903438
103. Centers for Disease Control and Prevention. Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes [Internet]. [cited 2021 Apr 7]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html
104. Viegas C, Coggins AM, Faria T, Caetano LA, Gomes AQ, Sabino R, et al. Fungal burden exposure assessment in podiatry clinics from Ireland. Int J Environ Health Res [Internet]. 2018;28(2):167–77. Available from: https://www.tandfonline.com/doi/full/10.1080/09603123.2018.1453053
105. Fernández-de-Mera IG, Rodríguez del-Río FJ, de la Fuente J, Pérez-Sancho M, Hervás D, Moreno I, et al. Detection of environmental SARS-CoV-2 RNA in a high prevalence setting in Spain. Transbound Emerg Dis [Internet]. 2020;(August):1–6. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.13817
106. Viegas C, Almeida B, Monteiro A, Paciência I, Rufo J, Aguiar L, et al. Exposure assessment in one central hospital: A multi-approach protocol to achieve an accurate risk characterization. Environ Res [Internet]. 2020;181(108947). Available from: https://www.sciencedirect.com/science/article/pii/S0013935119307443?via%3Dihub
107. Viegas C, Ramalho I, Alves M, Faria T, Caetano LA, Viegas S. Electrostatic dust cloth: A new sampling method for occupational exposure to bioaerosols. Review of literature. SHO-2017 – Int Symp Occup Saf Hyg [Internet]. 2017;(April):39–41. Available from:

https://www.researchgate.net/publication/316438445_Electrostatic_dust_cloth_-_A_new_sampling_method_for_occupational_exposure_to_bioaerosols

108. Lednicky JA, Lauzard M, Fan ZH, Jutla A, Tilly TB, Gangwar M, et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. Int J Infect Dis [Internet]. 2020;100:476–82. Available from: https://www.sciencedirect.com/science/article/pii/S1201971220307396
109. Ramos CA, Viegas C, Verde SC, Wolterbeek HT, Almeida SM. Characterizing the fungal and bacterial microflora and concentrations in fitness centres. Indoor Built Environ [Internet]. 2016;25(6):872–82. Available from: https://journals.sagepub.com/doi/abs/10.1177/1420326X15587954
110. Almeida-Silva M, Almeida SM, Pegas PN, Nunes T, Alves CA, Wolterbeek HT. Exposure and dose assessment to particle components among an elderly population. Atmos Environ [Internet]. 2015;102:156–66. Available from: https://www.sciencedirect.com/science/article/pii/S1352231014009352
111. Viegas C, Almeida-Silva M, Gomes AQ, Wolterbeek HT, Almeida SM. Fungal contamination assessment in portuguese elderly care centers. J Toxicol Environ Heal - Part A Curr Issues [Internet]. 2014;77(1–3):14–23. Available from: https://www.tandfonline.com/doi/full/10.1080/15287394.2014.861336
112. Pinheiro AC, Viegas C, Viegas S, Veríssimo C, Brandão J, MacEdo MF. Indoor air quality in portuguese archives: A snapshot on exposure levels. J Toxicol Environ Heal - Part A Curr Issues [Internet]. 2012;75(22–23):1359–70. Available from: https://www.tandfonline.com/doi/full/10.1080/15287394.2012.721168
113. Monteiro A, Almeida B, Paciência I, Rufo JC, Ribeiro E, Carolino E, et al. Bacterial contamination in health care centers: Differences between urban and rural settings. Atmosphere (Basel) [Internet]. 2021;12(4). Available from: https://www.mdpi.com/2073-4433/12/4/450/htm
114. Viegas C, Dias M, Monteiro A, Faria T, Lage J, Carolino E, et al. Bioburden in sleeping environments from Portuguese dwellings. Environ Pollut [Internet]. 2021;273:116417. Available from: https://www.sciencedirect.com/science/article/pii/S0269749120371062
115. Canha N, Alves AC, Marta CS, Lage J, Belo J, Faria T, et al. Compliance of indoor air quality during sleep with legislation and guidelines – A case study of Lisbon dwellings. Environ Pollut [Internet]. 2020;264. Available from: https://www.sciencedirect.com/science/article/pii/S0269749120309015
116. Viegas C, Faria T, Pacífico C, Dos Santos M, Monteiro A, Lança C, et al.

Microbiota and Particulate Matter Assessment in Portuguese Optical Shops Providing Contact Lens Services. Healthcare [Internet]. 2017;5(2):24. Available from: https://www.mdpi.com/2227-9032/5/2/24/htm

117. Srikanth P, Sudharsanam S, Steinberg R. Bio-Aerosols in Indoor Environment: Composition, Health Effects and Analysis. Indian J Med Microbiol [Internet]. 2008;26(4):302–12. Available from: https://www.sciencedirect.com/science/article/pii/S0255085721018053
118. Liofilchem. Dichloran Glycerol (DG18) Agar Base [Internet]. 2016 [cited 2021 Apr 16]. Available from: http://www.liofilchem.net/login/pd/ts/620238_TS.pdf
119. 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 [Internet]. 2011;2(1):15–20. Available from: https://www.researchgate.net/publication/233748672_Assessment_of_fungal_contamination_in_a_group_of_Lisbon%27s_Gymnasiumswith_a_swimming_Pool
120. Reboux G, Bellanger AP, Roussel S, Grenouillet F, Sornin S, Piarroux R, et al. Indoor mold concentration in Eastern France. Indoor Air [Internet]. 2009;19(6):446–53. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1600-0668.2009.00598.x
121. Adams RI, Miletto M, Taylor JW, Bruns TD. The diversity and distribution of fungi on residential surfaces. PLoS One [Internet]. 2013;8(11). Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0078866
122. Viegas C, Santos P, Almeida B, Monteiro A, Carolino E, Gomes AQ, et al. Electrostatic dust collector: a passive screening method to assess occupational exposure to organic dust in primary health care centers. Air Qual Atmos Heal [Internet]. 2019;12(5):573–83. Available from: https://link.springer.com/article/10.1007/s11869-018-0650-9
123. Viegas C, Twarużek M, Dias M, Almeida B, Carolino E, Kosicki R, et al. Assessment of the microbial contamination of mechanical protection gloves used on waste sorting industry: A contribution for the risk characterization. Environ Res [Internet]. 2020;189(June). Available from: https://www.sciencedirect.com/science/article/pii/S0013935120307763
124. Viegas C, Dias M, Almeida B, Aranha Caetano L, Carolino E, Quintal Gomes A, 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 [Internet]. 2020;102:856–67. Available from: https://www.sciencedirect.com/science/article/pii/S0956053X19307421

125. Centers for Disease Control and Prevention. Antifungal Resistance [Internet]. 2020 [cited 2021 Jun 20]. Available from: https://www.cdc.gov/fungal/antifungal-resistance.html
126. MacNeil L, Kauri T, Robertson W. Molecular techniques and their potential application in monitoring the microbiological quality of indoor air. Can J Microbiol [Internet]. 1995;41(8):657–65. Available from: https://cdnsciencepub.com/doi/10.1139/m95-091?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub 0pubmed
127. Viegas C, Faria T, Carolino E, Sabino R, Gomes AQ, Viegas S. Occupational exposure to fungi and particles in animal feed industry. Med Pr [Internet]. 2016;67(2):143–54. Available from: http://medpr.imp.lodz.pl/Occupational-exposure-to-fungi-and-particles-in-one-Portuguese-animal-feed-industry-a-preliminary-study,60085,0,2.html
128. Condalab. Malt Extract Agar [Internet]. 2019 [cited 2021 Apr 14]. Available from: https://www.condalab.com/int/en/dehydrated-culture-media/32-11458-malt-extract-agar.html
129. Liofilchem. Malt Extract Agar. 2004;(3). Available from: http://www.liofilchem.net/login/pd/ts/610173_TS.pdf
130. Williams J, Clarkson JM, Mills PR, Cooper RM. A selective medium for quantitative reisolation of Trichoderma harzianum from Agaricus bisporus compost. Appl Environ Microbiol [Internet]. 2003;69(7):4190–1. Available from: https://journals.asm.org/doi/full/10.1128/AEM.69.7.4190-4191.2003
131. Merck. Media for Brewery, Winery and Fermentations [Internet]. [cited 2021 Apr 14]. Available from: https://www.sigmaaldrich.com/analytical-chromatography/microbiology/microbiology-products.html?TablePage=17996750
132. Neogen. DICHLORAN GLYCEROL (DG-18) AGAR BASE (7592) [Internet]. [cited 2021 Apr 16]. Available from: http://biotrading.com/assets/productinformatie/acumedia/tds/7592.pdf
133. Liofilchem. Tryptic Soy Agar [Internet]. 2020 [cited 2021 Apr 16]. Available from: http://www.liofilchem.net/login/pd/ifu/10037_IFU.pdf
134. Scientific T. Dehydrated Culture Media - TRYPTONE SOYA AGAR (Casein soya bean digest agar) EP/USP/JP/BP [Internet]. [cited 2021 Apr 16]. Available from:

http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0131&cat=&c=UK&lang=EN

135. Merck. Technical Data Sheet GranuCult™ Tryptic Soy Agar.
136. HiMedia Laboratories. Technical Data - Violet Red Bile Agar M049 [Internet]. 2019 [cited 2021 Apr 16]. Available from: https://www.himedialabs.com/TD/M049.pdf
137. Neogen. Technical Specification Sheet - Violet Red Bile Agar (NCM0025). 2019.
138. Scientific T. Oxoid™ Violet Red Bile Agar [Internet]. [cited 2021 Apr 16]. Available from: https://www.thermofisher.com/order/catalog/product/CM0107B#/CM0107B
139. Liofilchem. Sabouraud Dextrose Agar [Internet]. 2019 [cited 2021 Apr 17]. Available from: http://www.liofilchem.net/login/pd/ifu/10035_IFU.pdf
140. Google Maps. Google Maps (Portugal) [Internet]. 2021 [cited 2021 Jul 27]. Available from: https://www.google.pt/maps/place/Portugal/@39.5474739,-7.8589993,7z/data=!4m5!3m4!1s0xb32242dbf4226d5:0x2ab84b091c4ef041!8m2!3d39.399872!4d-8.224454?hl=pt-PT

8. Appendix

8.1. Appendix 8.1 – Description of culture media used

Table 8.1 – Description of each culture media used.

Culture media	Description
MEA	MEA is a nutrient-rich medium for the cultivation of environmental fungi (46). It is particularly suitable for isolating, cultivating and enumerating of yeasts and molds as it contains a high concentration of maltose and other saccharides as energy sources (46,128,129). Dextrin and glycerin (added during the preparation of medium) are the carbon sources, and peptone is a nitrogen source (128). Chloramphenicol is an antibiotic added to the medium that prevent the growth of bacteria and actinomycetes (130). Agar is the solidifying agent (128). The alcohol content and the acid pH of the medium having also inhibitory effects that allow the optimal growth of yeasts and fungi while restricting bacterial growth (46,128,129,131). In summary, MEA allows fast-growing fungal species to grow on its surface, especially due to high sugar content and water activity (46).
DG18	DG18 is firstly used for the enumeration of xerophilic molds and osmophilic yeasts (46). Enzymatic digest of casein provides amino acids, carbon, nitrogen, vitamins and minerals for organisms growth (118). Glucose is included as energy source (118). Monopotassium phosphate is a buffering agent (118). Magnesium sulfate provides divalent cations and sulfur and it is an inorganic salt used to stimulate fungal growth and sporulation (118,132). Agar is the solidifying agent (118). Glycerol is included to lower the water activity (aW) of the medium from 0.999 to 0.95 and provide an additional carbon source (46,118). Besides that, glycerol has the advantage of culture a wider range of xerophilic fungi when compared to chloride and sugars, which have traditionally been used to formulate media of reduced aW (46). It has been suggested that this medium is a better alternative for colony counting and to obtain higher diversity of genera, since contains dichloran, which is an anti-fungal agent that inhibits bacterial growth and restrict the spread of more-rapidly growing fungi, such as fungi belonging to the Mucorales order (including *Rhizopus* and *Mucor* genera) and restricts the colony size of other genera (46,118). This restrictive characteristic makes the medium especially suitable for enumeration because it allows for the unobscured growth of organisms that ordinarily form small colonies (46). Thus, DG18 allows the identification of a more diverse fungal flora, but excludes fungi requiring high water activity to grow (46).
TSA	TSA is a non-selective isolation medium used for the growth of bacteria (133). The

	combination of the two peptones - pancreatic digest of casein and papaic digest of soy bean - provides a high nutrition for organisms growth by supplying organic nitrogen, amino acids and longer-chained peptides (133–135). Sodium chloride maintains osmotic balance in the medium and agar is the solidifying agent (133,135).
VRBA	VRBA is used for selective isolation, detection and enumeration of coli aerogenes bacterial group (136–138). The coliform group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-spore forming bacilli (136,137). Coliforms ferment lactose and form acid and gas at 35°C within 48 hours (136,137). Members of Enterobacteriaceae comprise the majority of the group, but other lactose fermenting organisms may also be included (136,137). This medium relies on the use of the selective inhibitory components crystals violet, bile salts and neutral red that suppress the growth of non-target Gram-positive bacteria (136,138). Organisms, which rapidly fermenting the lactose (coliforms), produce red-purple colonies surrounded by purple halos (136,138). Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones (136,138). Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients (136). Lactose is the fermentable carbohydrate, utilization of which leads to the production of acids (136). Neutral red indicator also detects the acidity so formed (136). Sodium chloride maintains the osmotic equilibrium (136).
SAB	SAB is a non selective isolation medium used for the growth and maintenance of pathogenic and non-pathogenic fungi from clinical and nonclinical specimens (139). It is also used for recovery and total counting of yeasts and fungi in environmental monitoring (139). Pancreatic digest of casein and peptic digest of animal tissue provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth (139). Dextrose is an energy source (139). Agar is the solidifying agent (139). The high concentration of dextrose and the acidic pH of the medium permit selectivity of fungi (139).

8.2. Appendix 8.2 – Scientific technical report

1. Índice

2. Índice de tabelas

3. Introdução

O presente relatório refere-se à avaliação da exposição/ contaminação realizada de julho a dezembro do ano 2020, no ambiente ocupacional do [redacted]

As amostras recolhidas foram processadas no laboratório de microbiologia ambiental da Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL) entre 29 a julho de 2020 a 1 de julho de 2021, de acordo com os protocolos em vigor para a análise de amostras ambientais.

Esta avaliação decorre das actividades de investigação da área de investigação Ambiente e Saúde do *Health and Technology Research Center* (H&TRC) (https://htrc.estesl.ipl.pt/htrc/).

4. Contexto

O presente relatório reporta os resultados provenientes de amostras recolhidas no contexto ocupacional [redacted] por investigadores do laboratório de microbiologia ambiental da ESTeSL.

As amostras recolhidas refletem a diversidade da contaminação microbiana e foram obtidas em dez instalações do [redacted], o que incluiu o edifício da Presidência, os Serviços de Ação Social e oito unidades orgânicas dedicadas ao ensino, nomeadamente a [redacted] A avaliação da contaminação microbiológica nestes locais foi efetuada no âmbito do projeto "IPL Momento Zero", que teve como objetivo assegurar a segurança na retoma letiva em situação pandémica, tendo este projeto incluído a deteção da presença do *SARS-CoV-2* nos locais descritos acima. Além da deteção do vírus pandémico, foi efetuada também a avaliação da contaminação por fungos e bactérias nos mesmos locais de amostragem.

5. Dados

Na tabela 1 encontram-se os dados relativos à entidade onde foi realizada a avaliação e aos quais diz respeito os resultados apresentados no presente Relatório Técnico-Científico.

Tabela 1 - Dados gerais

Identificação da amostra	
Projeto de investigação/ Cliente externo:	Avaliação da contaminação microbiológica nas Unidades orgânicas do [redacted]
Data de início:	22/07/2020
Duração da análise (meses ou dias):	12 meses
Data de conclusão do relatório:	27/07/2021
Nº do relatório:	1/2021
Trabalho de campo realizado por:	Carla Viegas, Marta Dias, Raquel Pimenta e Blanca Gomes
Colheitas ambientais realizadas por:	Marta Dias, Raquel Pimenta e Blanca Gomes
Trabalho laboratorial realizado por:	Marta Dias, Raquel Pimenta e Blanca Gomes
Relatório realizado por:	Carla Viegas e Raquel Pimenta com o apoio de Sandra Ferreira e Edlane Silva

6. Siglas e Definições

Bactérias Gram-negativas: Bactérias constituídas por uma dupla camada de membranas, sendo na externa expresso um potente indutor lipopolissacarídeo (LPS) (Oliveira e Reygaert, 2020), são produtoras de endotoxinas (Maldonado et al., 2016 e Bertani & Ruiz, 2018). Estas bactérias não retêm o corante primário utilizado na coloração de Gram, retendo o segundo corante ou contracorante usualmente Fucsina ou Safranina (Tripathi, e Sapra, 2020).

Bactérias: Organismos procarióticos unicelulares classificados clinicamente com base nas suas formas (Sefton, 2019).

Bioaerossóis: Partículas de aerossol de origem biológica (por exemplo, bactérias, fungos, esporos de fungos, pólen, fragmentos de biofilme, ... (Liao et al., 2021).

Colónia: Agrupamento de indivíduos de uma mesma espécie, onde os organismos associados se encontram unidos através de um substrato comum (Tortura et al., 2009).

DG18: Dichloran glycerol chloramphenicol (Millipore 2021).

EDC: Electrostatic Dust Cloth (Viegas et al., 2016a).

Endotoxinas: Componente lipopolissacarídeo da célula da bactéria Gram-negativa que é liberada durante o crescimento celular ativo e após a lise celular (Stetzenbach, 2009)

Esporos: Estruturas metabolicamente inativas e tolerantes ao stress ambiental (ao contrário das células vegetativas). Importante meio de dispersão de organismos para outros habitats (Setlow, 2006).

Fungos: Organismos eucariotas que possuem um núcleo definido, que contém material genético (DNA) envolto por um envelope denominado de membrana nuclear (Tortora et al., 2009).

Fungos ambientais ou geofílicos: Compreendem os fungos isolados habitualmente no solo, que só ocasionalmente são patogénicos ao homem ou outros animais (Ferreira, 2000).

Fungos potencialmente patogénicos: São fungos que causam doença apenas em determinadas circunstâncias, a maior parte das vezes envolvendo debilitação do indivíduo (Caio et al., 2013).

H&TRC: Health and Technology Research Center.

Humidade relativa: Principal condicionante ambiental para a proliferação de ácaros e fungos em ambientes interiores. É recomendado a humidade relativa em ambientes interiores se mantenha abaixo dos 75% para prevenir a proliferação destes organismos (WHO 2009)

Leveduras: Fungos unicelulares, não filamentosos (Ferreira, 2000).

Local de referência: Local de captação de ar novo (Exterior das instalações) (APA).

Matéria Particulada (*Particulate Matter*): Mistura de partículas sólidas e gotículas líquidas encontradas no ar. Algumas partículas, como poeira, sujeira, fuligem ou fumaça, são grandes ou escuras o suficiente para serem vistas a olho nu. Outros são tão pequenos que só podem ser detetados com um microscópio eletrónico (U.S. EPA, 2020).

MEA: Malt Extract Agar (Millipore 2021).

Micotoxinas: Metabolitos secundários tóxicos produzidos por fungos filamentosos que contaminam várias colheitas de alimentos e rações, apresentando sérios riscos à saúde humana e animal (Pankaj et al., 2018).

Partículas (PM_{10}): partículas inaláveis, com diâmetros geralmente de 10 micrômetros ou menores (U.S.EPA, 2020).

Partículas ($PM_{2.5}$): partículas finas inaláveis, com diâmetros que geralmente são 2,5 micrômetros e menores (U.S. EPA, 2020).

PMC: Matéria particulada – valor mássico (Soppa et al., 2014).

PNC: Contagem do número de partículas (Soppa et al., 2014).

Proliferação: Rápida reprodução de microrganismos (Eduard, 2009).

qPCR: Quantitative Polymerase Chain Reaction (Forero et al., 2019).

Qualidade do ar interior (QAI): É o conjunto de características físicas, químicas e biológicas dos espaços internos do edificado ex: escritórios, salas, gabinetes, quartos. Não contempla o espaço interior industrial (APA, 2009).

Temperatura: Factor ambiental que afeta todos os organismos terrestres, devido à relação das taxas de reações bioquímicas e processos biológicos com a temperatura. O aumento das taxas de reações bioquímicas é potenciado com o aumento da temperatura (Klepsatel et al., 2019)

TSA: Tryptone Soya Agar (Millipore 2021).

UFC: Unidades formadoras de colónias, geralmente abreviado como UFC, referem-se às colónias individuais de microrganismos. Por exemplo, uma colónia de bactérias ou de leveduras refere-se a uma massa de células individuais do mesmo organismo, que crescem em conjunto. A colónia é um grupo de células que crescem juntas. Unidades formadoras de colónias são utilizadas como uma medida do número de microrganismos presentes numa amostra (Tortora et al., 2009).

VRBA: Violet Red Bile Agar (Millipore 2021).

7. Abordagem metodológica

Os locais de colheita em cada instalação foram previamente selecionados pelos Serviços de Saúde Ocupacional com base em critérios definidos, de modo a priorizar as áreas de trabalho mais críticas para a deteção do *SARS-CoV-2*. Os critérios aplicados foram: 50-60% de ocupação das instalações, atividades realizadas ou com base nos resultados da vigilância serológica dos trabalhadores.

Foram recolhidas amostras de superfícies geralmente utilizadas pelos trabalhadores em escritórios, salas de atendimentos, receção, auditórios, espaços de refeição, casas de banho, bibliotecas, salas de aula e ginásios. A amostragem foi efetuada no período entre o verão e o outono e, maioritariamente, durante a manhã e durante o curso das atividades normais, com exceção da [redacted] que foi realizada em dois dias, sendo um deles no período da tarde.

8. Equipamentos e Materiais

A Tabela 2 apresenta as informações respeitantes à estratégia de amostragem e as variáveis microbiológicas analisadas.

Tabela 2 – Procedimentos utilizados, parâmetros microbiológicos analisados e material utilizado

Método de colheita	Variável	Equipamento	Marca	Material associado
Colheita de amostra-Ar (Impinger)	Fungos (UFC/m³)	Coriolis µ air sampler	Bertin Technologies	Solução tampão; Parafilm; Luvas; Álcool.
Colheita de superfície	Fungos (UFC/m²) Bactérias (UFC/m²)	X	X	Zaragatoas; Forma metálica para delimitação de área a amostrar; Soro fisiológico; Luvas; Álcool; Algodão.

9. Resultados

Na tabela 3 encontram-se os locais com maior contaminação microbiana por Unidade Orgânica, obtidos através de microbiologia clássica.

No que concerne aos locais com maior contaminação microbiana o estúdio Átrio C-1 da unidade [redacted] foi o único local com elevada quantidade de bactérias totais e Gram-negativas. O departamento de compras (1ºandar) da unidade Presidência e, ainda, o espaço "comida de casa" da unidade [redacted] foram os locais com maior contaminação de bactérias totais. Encontrou-se uma maior prevalência de bactérias Gram-negativas na oficina de cerâmica da [redacted] e nos serviços académicos da [redacted]

Em relação à contaminação fúngica, no meio MEA destacam-se a sala de coro da [redacted] e o balneário/vestiário da [redacted]. No meio DG18 é de salientar a sala de professores 2 do [redacted] e o gabinete de comunicação da Presidência.

Tabela 3 – Locais com maior contaminação microbiana por Unidade Orgânica em UFC/m^2.

Unidade	Local amostrado	Bactérias totais	Bactérias Gram-negativas	Fungos (MEA)	Fungos (DG18)
Serviços de Ação Social	Casa de Banho	$3{,}20 \times 10^{+04}$			
Serviços de Ação Social	Sala de Espera/Sala de Aulas			$1{,}00 \times 10^{+04}$	$9{,}00 \times 10^{+03}$
Presidência	Departamento de Compras (1º andar)	$5{,}62 \times 10^{+05}$			
Presidência	Gabinete de Comunicação			$5{,}70 \times 10^{+04}$	$7{,}70 \times 10^{+04}$
[redacted]	Gabinete Docente (1.16)	$1{,}94 \times 10^{+05}$			
	Impressora (1º andar)			$1{,}20 \times 10^{+04}$	$1{,}20 \times 10^{+04}$
	Serviços Financeiros	$1{,}60 \times 10^{+04}$			
	Sala de Professores 2			$1{,}00 \times 10^{+04}$	$1{,}61 \times 10^{+05}$
	Biblioteca	$1{,}21 \times 10^{+05}$			
	Laboratório de Química orgânica		$1{,}50 \times 10^{+04}$		$1{,}50 \times 10^{+04}$
	Bar			$3{,}00 \times 10^{+03}$	

Unidade	Local amostrado	Bactérias totais	Bactérias Gram-negativas	Fungos (MEA)	Fungos (DG18)
[redacted]	Grande Auditório	4,48 x10^{+05}		1,00 x10^{+03}	
	Bar		1,00 x10^{+03}		
	Biblioteca		1,00 x10^{+03}		
	Refeitório			1,00 x10+03	
	Oficina				4,00 x10^{+03}
	Sala de Dança	4,28 x10^{+05}			
	Oficina de Cerâmica		7,00 x10^{+03}		
	Balneário/ Vestiário			5,90 x10^{+04}	6,70 x10^{+04}
	Sala de Música	4,81 x10^{+05}			
	Serviços Académicos		6,00 x10^{+03}		
	Sala de coro			6,80 x10^{+04}	
	Grande Auditório				2,80 x10^{+04}
	Espaço comida de casa	5,52 x10^{+05}			
	Sala de estudo		2,00 x10^{+03}		2,00 x10^{+04}
	Auditório			1,70 x10^{+04}	
	Estúdio Átrio C -1	1,17 x10^{+05}	3,00 x10^{+03}		
	Sala de professores			1,40 x10^{+04}	1,40 x10^{+04}

Para uma melhor caracterização da contaminação fúngica, foram identificadas as espécies fúngicas presentes nas superfícies amostradas, conforme demonstrado na Tabela 4.

No total foram identificadas 13 espécies fúngicas diferentes nos meios de cultura MEA e DG18. O fungo mais prevalente em ambos os meios de cultura foi *Cladosporium* sp. (47,36% MEA; 32,33% DG18). Além disso, no meio de cultura MEA foi também identificado com frequência o *Penicillium* sp. (40,94%), seguido de *Aspergillus* sp. (3,21%). No meio de cultura DG18, observou-se também uma maior predominância de *Aureobasidium* sp. (28,97%) e *Penicillium* sp. (20,67%).

Em relação ao género *Aspergillus*, obtiveram-se 6 *sections* distintas, sendo que *Candidi* (35,29% no MEA) e *Nidulantes* (92,77% no DG18) foram as *sections* com maior distribuição no meio MEA e DG18, respetivamente.

Tabela 4 - Resultados obtidos relativos à distribuição fúngica em UFC/m³.

Meio de cultura MEA		**Meio de cultura DG18**	
Espécie fúngica	**Contagem total (UFC/m³; %)**	**Espécie fúngica**	**Contagem total (UFC/m³; %)**
Cladosporium sp.	2,51 x10^{+05}; 47,36	*Cladosporium* sp.	1,83 x10^{+05}; 32,33
Penicillium sp.	2,17 x10^{+05}; 40,94	*Aureobasidium* sp.	1,64 x10^{+05}; 28,98
Aspergillus sp.	1,70 x10^{+04}; 3,21	*Penicillium* sp.	1,17 x10^{+05}; 20,67
Rhizopus sp.	1,40 x10^{+04}; 2,64	*Aspergillus* sp.	8,30 x10^{+04}; 14,66
Aureobasidium sp.	1,20 x10^{+04}; 2,26	*Chrysosporium* sp.	1,10 x10^{+04}; 1,94
Alternaria sp.	8,00 x10^{+03}; 1,51	*Fusarium* sp.	3,00 x10^{+03}; 0,53
Chrysosporium sp.	5,00 x10^{+03}; 0,94	*Chrysonilia* sp.	2,00 x10^{+03}; 0,35
Phoma sp.	3,00 x10^{+03}; 0,57	*Mucor* sp.	2,00 x10^{+03}; 0,35
Acremonium sp.	2,00 x10^{+03}; 0,38	*Acremonium* sp.	1,00 x10^{+03}; 0,18
Chrysonilia sitophila	1,00 x10^{+03}; 0,19		
Total	5,30 x10^{+05}; 100		5,66 x10^{+05}; 100

Nas figuras 1 e 2 pode ser observada a distribuição da contaminação microbiana por local amostrado. Na figura 1, pode ser observada a distribuição da carga microbiana total, onde é visível a forte prevalência de bactérias totais e na figura 2 pode ser observada a mesma distribuição, mas sem a variável de bactérias totais.

Figura 1 - Resumo de resultados por locar de amostragem.

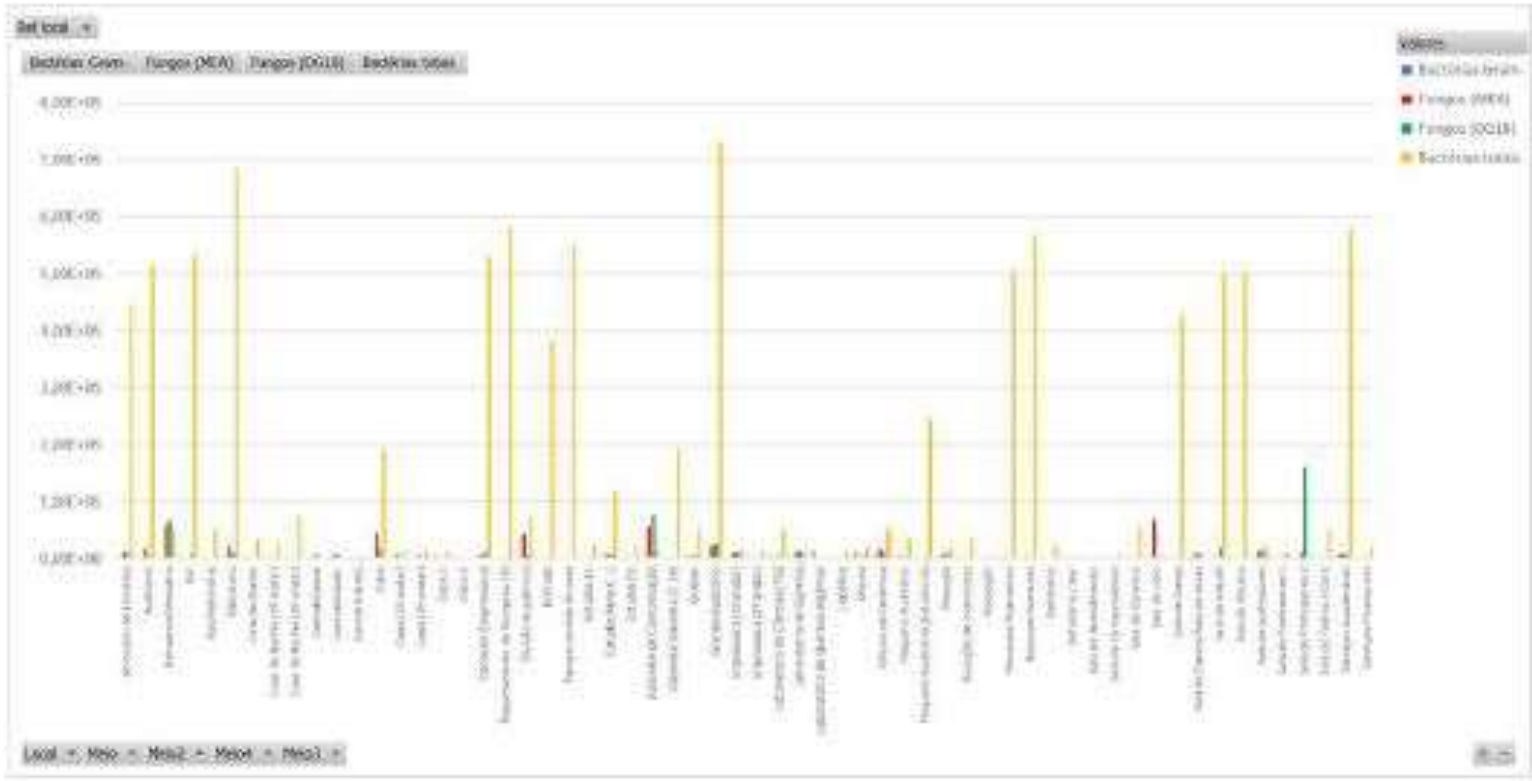
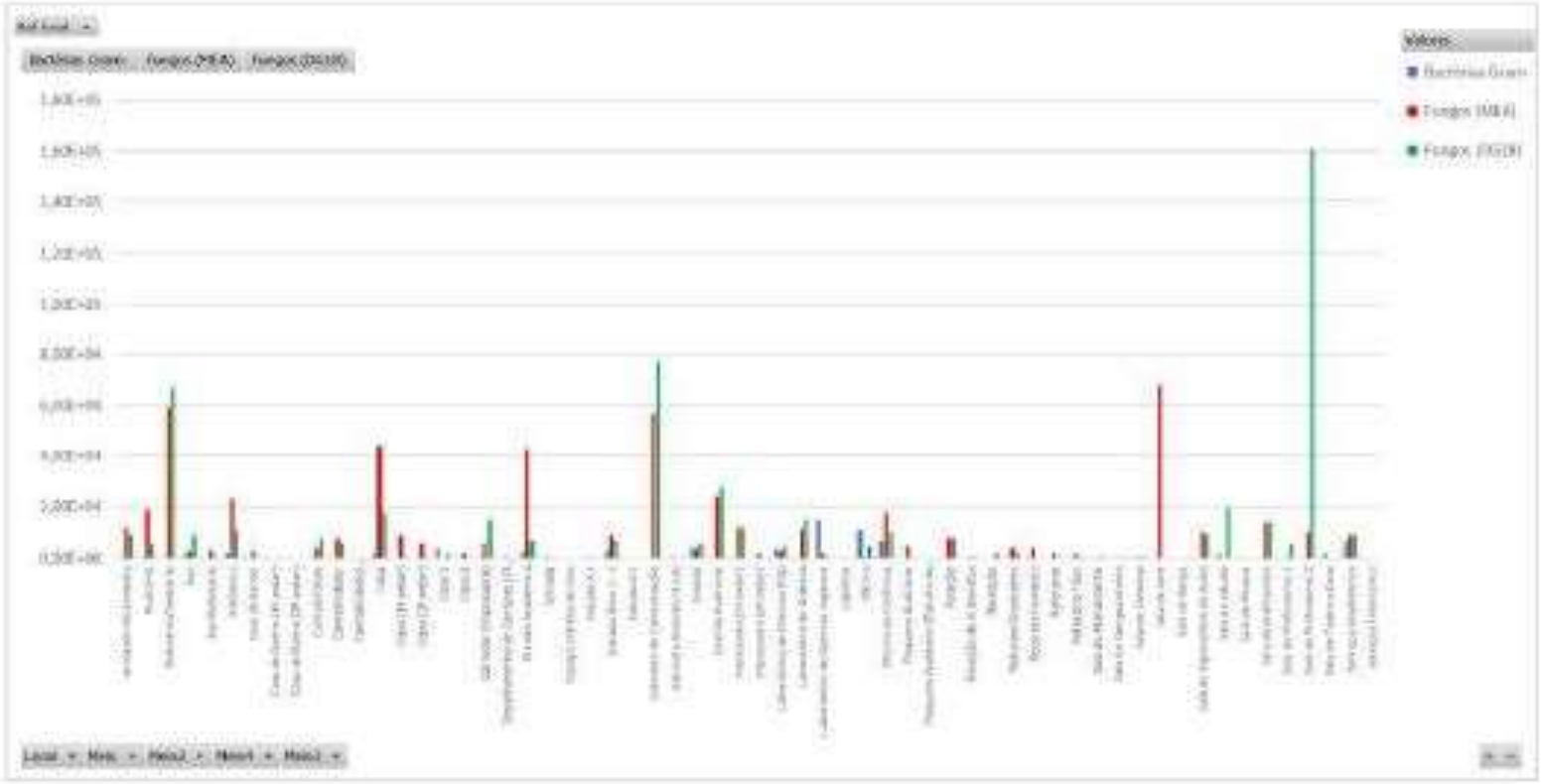


Figura 2 - Resumo de resultados por local de amostragem de Bactérias Gram-negativas, Fungos (MEA), Fungos (DG18).



A identificação molecular de *Aspergillus* section *Fumigati* através de PCR em tempo real foi efetuada nas amostras de ar e de superfície recolhidas, devido à sua relevância clínica. Não foi detetado nas amostras de ar, no entanto foi detetada em 2 amostras de superfície (2,22%, 2 em 90 amostras).

10. Discussão de resultados

Apesar da não existência de limites nacionais quanto à presença de microrganismos em superfícies é importante salientar que a aerossolização das espécies que se encontram nas superfícies dependerá da influência de variáveis ambientais e de características fúngicas (Roussel et al., 2008). Os resultados das superfícies são essenciais para alcançar a caracterização e avaliação de contaminação por fungos e bactérias, e podem ser utilizados para identificar as fontes de contaminação (Stetzenbach et al., 2004; Klánová e Hollerová 2003).

Foi possível verificar uma coexistência de bactérias e fungos nos locais amostrados. Em relação às bactérias, conforme espectável, foi evidente uma menor concentração de bactérias Gram-negativas do que de bactérias totais, pois estas últimas representam a soma de bactérias Gram-positivas e Gram-negativas. Além disso, as Gram-negativas são tammbém mais sensíveis às variavés ambientais, não sendo tão persistentes no ambiente.

Verificou-se uma maior prevalência de bactérias do que de fungos, tal como aconteceu num estudo em que foram analisadas as superfícies de várias instalações de uma universidade (Haleem et al., 2013).

Relativamente à contaminação bacteriana foi possível verificar que as bactérias totais se encontram amplamente distribuídas em todos ambientes estudados, sendo os locais com maior contaminação por unidade orgânica descritos abaixo:

- Presidência - Recursos humanos (5,6 x 10^5 UFC/m²);
- [redacted] Sala de Música (4,8 x 10^5 UFC/m²);
- [redacted] Grande Auditório (4,5 x 10^5 UFC/m²);
- [redacted] - Sala de Dança (4,3 x 10^5 UFC/m²);
- [redacted] - Gabinete Docente (1.16) (1,9 x 10^5 UFC/m²);
- [redacted] Biblioteca (1,2 x 10^5 UFC/m²);
- [redacted] - Casa de Banho (3 x 10^4 UFC/m²);
- [redacted] Estúdio Átrio C -1 (1,2 x 10^5 UFC/m²);

- [redacted]- Sala de estudo (2,0 x 10^4 UFC/m^2);
- [redacted]-Serviços Financeiros (1,8 x 10^4 UFC/m^2).

Quanto às bactérias Gram-negativas, estas não foram detetadas em grande parte dos locais amostrados, sendo os locais com maior contaminação por unidade descritos abaixo:

- [redacted]aboratório de Química orgânica (1,5 x10^4 UFC/m^2);
- [redacted]- Oficina de Cerâmica (7,0 x10^3 UFC/m^2);
- [redacted] Serviços Académicos (6,0*10^3 UFC/m^2);
- [redacted]Estúdio Átrio C -1 (3,0 x10^3 UFC/m^2);
- [redacted] Sala de estudo (2,0 x10^3 UFC/m^2);
- [redacted] Biblioteca e Bar (1,0 x10^3 UFC/m^2)

As superfícies com maior utilização por parte de diferentes pessoas, como maçanetas de metal e bancadas de laboratório, têm sido reportadas com elevada contaminação bacteriana (Ross & Neufeld 2015; Zulfakar et al. 2019). Uma inadequada higiene pessoal pode aumentar a diversidade de microrganismos nas mãos e, por extensão, em superfícies de contacto (Ross & Neufeld 2015).
A maioria das bactérias encontradas no interior de edifícios pertence à microflora normal da pele, boca e nariz, e são continuamente emitidas pelas pessoas, acumulando-se em ambientes fechados (Zhu et al. 2003; APA, 2010; Moldoveanu et al. 2015). Estas bactérias geralmente não constituem perigo para a saúde (APA, 2010). Por outro lado, a existência de bactérias Gram-negativas, deve ser considerada, pois estas bactérias podem produzir endotoxinas, que são toxinas prejudiciais à saúde, provocando problemas respiratórios (Moldoveanu et al. 2015; WHO, 2009).

Relativamente aos resultados da contaminação fúngica das superfícies, no meio de cultura MEA, os locais com maior contaminação por unidade são descritos abaixo:

- [redacted] Sala de Coro (6, 80 x10^4 UFC/m^2);
- [redacted]- Balneário/ Vestiário (5,90 x10^4 UFC/m^2);
- [redacted]- Gabinete de comunicação (5,70 x10^4 UFC/m^2);
- [redacted]Auditório (1,7 x10^4 UFC/m^2);
- [redacted] Sala dos professores (1,4 x10^4 UFC/m^2);
- [redacted]- Impressora (1º andar) (1,2 x10^4 UFC/m^2);
- [redacted] Laboratório de Química Inorgânica (1,1 x10^4 UFC/m^2);
- [redacted]- Sala de Espera/Sala de Aulas (1,0 x10^4 UFC/m^2);

- [redacted] Sala de Professores 2 (1,0 x10^4 UFC/m^2);
- [redacted] Refeitório e Grande Auditório (1,0 x10^4 UFC/m^2).

Em relação ao meio de cultura DG18, os locais com maior contaminação por unidade estão descritos abaixo:

- [redacted] Sala dos professores 2 (1,6 x10^5 UFC/m^2);
- [redacted] - Gabinete de comunicação (7,70 x10^4 UFC/m^2);
- [redacted] Balneário/ vestiário (6, 70 x10^4 UFC/m^2);
- [redacted] Grande Auditório (2,8 x10^4 UFC/m^2);
- [redacted] Sala de estudo (2,0 x10^4 UFC/m^2);
- [redacted] Laboratório de Química Inorgânica (1,5 x10^4 UFC/m^2);
- [redacted] Sala dos professores (1,4 x10^4 UFC/m^2);
- [redacted] - Impressora (1º andar) (1,2 x10^4 UFC/m^2);
- [redacted] Sala de Espera/Sala de Aulas (9 x10^3 UFC/m^2);
- [redacted] Oficina (4,0x10^3 UFC/m^2).

Para ambos os meios de cultura (DG18 e MEA), os resultados poderão dever-se à ineficácia nos procedimentos de limpeza e de ventilação dos locais (Viegas et al., 2015; Portaria 353-A de 2013 de 4 de dezembro). A temperatura e a humidade, também podem promover o crescimento de fungos e a produção de micotoxinas (Boudra *et al.*, 2005).

Verifica-se que os fungos cresceram em ambos os meios em todos os locais, exceto em [redacted], isto poderá dever-se à capacidade de alguns fungos crescerem de forma diferente nos meios de cultura DG18 e MEA.

No meio de cultura MEA foi encontrada uma maior prevalência de *Cladosporium* sp. (47,36%), seguido de *Penicillium* sp. (40,94%) e *Aspergillus* sp. (3,21%). No meio de cultura DG18, o mais prevalente foi *Cladosporium* sp. (32,33%), seguido de *Aureobasidium* sp. (28,98%) e *Penicillium* sp. (20,67%).

Para uma melhor caracterização da contaminação fúngica, foram elencados os fungos mais prevalentes e os que apresentam relevância clínica e toxicológica, conforme demonstrado na tabela 5.

Tabela 5 - Fungos com maior prevalência e relevância clínica e toxicológica

Fungos com > prevalência nas amostras (métodos passivos) (Viegas et al., 2020)	*Cladosporium* sp., *Penicillium* sp., *Aureobasidium* sp., *Aspergillus* sp.
Fungos com relevância clínica (de acordo com o Decreto-Lei n.º 102-A/2020)	*Aspergillus* sp.
Fungos com relevância toxicológica (Serra, 2005)	*Aspergillus* sp., *Fusarium* sp., *Penicillium* sp.

Cladosporium sp., *Penicillium* sp., e *Aspergillus* sp., são fungos geralmente encontrados em ambientes interiores, incluindo em instituições de ensino superior (Stryjakowska-Sekulska, 2007).

Cladosporium sp. em ambientes interiores tem como origem principal fontes externas (Fukutomi & Taniguchi 2015). Devido ao tamanho reduzido dos seus esporos, são facilmente disseminados por longas distâncias (Bensch et al., 2012). É geralmente associado a rinite alérgica ou a lesões localizadas superficiais ou profundas, mas, raramente causam infeções disseminadas (Sandoval-Denis et al., 2015). Algumas espécies podem causar micose pulmonar alérgica (Bensch et al., 2012; Ziaee et al., 2018).

Penicillium sp. é mais comum no ar interior do que no ar exterior (Reboux et al., 2019). Encontram-se onde exista disponibilidade de matéria orgânica, podendo crescer em condições com muito pouca água (Ziaee et al., 2018). O aumento da humidade relativa contribui para concentrações elevadas de *Penicillium* (Reboux et al., 2019). Apesar de estarem distribuídos por diversos ambientes, geralmente não são associados a infeções para os humanos e animais (Egbuta et al., 2017). No entanto, algumas espécies podem causar infeções e outras doenças, como por exemplo, pneumonias, infeções urinárias e asma (Egbuta et al., 2017). Além disso, podem produzir micotoxinas como a ocratoxina A, considerada cancerígena (Egbuta et al., 2017).

Aspergillus sp. pode crescer em elevadas temperaturas e em ambientes húmidos (Sabino et al., 2019). Têm uma alta versatilidade nutricional e são capazes de utilizar diversos compostos orgânicos e substâncias como fonte de carbono (Sabino et al., 2019). O tamanho reduzido de esporos facilita a sua disseminação pelo ar (Sabino et al., 2019). Muitas espécies têm sido reportadas como agentes causadores de infeções oportunistas no homem (Egbuta et al., 2017). As doenças mais comuns associadas à exposição ocupacional a *Aspergillus* sp. são: aspergilose broncopulmonar alérgica,

rinossinusite, rinite e asma severa por sensibilização fúngica (Sabino et al., 2019). Além disso, algumas espécies são capazes de produzir micotoxinas, que são prejudiciais para a saúde (Egbuta et al., 2017).

Aureobasidium sp. é associado a problemas nos edifícios e também está relacionado com o desenvolvimento de pneumonite de hipersensibilidade e rinite alérgica (Stark et al., 2005).

A espécie *Fusarium* encontra-se amplamente distribuída no solo, água, sendo esta sua capacidade de difusão em diferentes ambientes atribuída à sua capacidade de crescer em diferentes substratos. Esta espécie pode causar infeções superficiais, assim como doenças do foro alérgico (Nucci & Anaissie, 2007). As espécies do género *Fusarium* são consideradas como indicadores de problemas de humidade ou de risco para a saúde (Goyer et al., 2001).

Apesar de espécies da section *Fumigati* terem sido identificadas em amostras de superfície através dos métodos baseados em cultura, a identificação molecular permitiu detetar a sua presença em duas amostras distintas, tal como aconteceu anteriormente noutros estudos (Viegas et al., 2016b; Viegas et al., 2021).

11. Recomendações

Em todas as instalações, os procedimentos adotados reportados foram limpeza e desinfeção normal. Os produtos de limpeza utilizados foram bactericidas e virucidas, lixívia e detergente multiusos. Apesar de ser evidente uma limpeza regular de todos os espaços, foi notório que a maioria das unidades e das superfícies amostradas apresentou contaminação microbiológica. De um modo geral, e considerando os resultados obtidos, deverão ser realizadas avaliações antes e depois da limpeza, de modo a verificar se os procedimentos e os produtos utilizados são os mais adequados.

Outro aspeto importante é garantir formação adequada aos trabalhadores sobre os métodos de limpeza mais eficazes para diminuir a quantidade de fungos e bactérias.

Além disso, uma regular monitorização do ar interior complementada com avaliações das superfícies nestes espaços, seria importante para garantir a saúde e o desempenho de todos os trabalhadores e dos estudantes.

11. Referências

- Agência Portuguesa do Ambiente (APA). (2009). Qualidade do Ar em Espaços Interiores - Guia técnico. Portugal: Agência Portuguesa do Ambiente.
- Agência Portuguesa do Ambiente (APA). (2010). Qualidade do Ar em Espaços Interiores- Guia Técnico. Portugal: Agência Portuguesa do Ambiente. Disponível em: http://apambiente.pt/_zdata/Divulgacao/Publicacoes/Guias e Manuais/manual QArInt_standard.pdf.
- Baatjies R., Meijster T., Heederik D., Sander I., Jeebhay M.F. (2014). Effectiveness of interventions to reduce flour dust exposures in supermarket bakeries in South Africa. Occup Environ Med, 71, 811–818.
- Bensch, K., Braun, U., Groenewald, J. Z., & Crous, P. W. (2012). The genus Cladosporium. Studies in mycology, 72(1), 1–401. https://doi.org/10.3114/sim0003.
- Bertani, B., & Ruiz, N. (2018). Function and Biogenesis of Lipopolysaccharides. EcoSal Plus, 8(1), 10.1128/ecosalplus.ESP-0001-2018. https://doi.org/10.1128/ecosalplus.ESP-0001-2018.
- Cailo, S., Billmyre, R. B., Heitman, J. (2013). Generators of phenotypic diversity in the evolution of pathogenic microorganisms. PLoS Pathog, 9(3), e1003181.
- Decreto-Lei nº 243/86 de 20/8 - Aprova o Regulamento Geral de Higiene e Segurança do Trabalho nos Estabelecimentos Comerciais, de Escritório e Serviços.
- Dionova, B., Mohammed, M.N., Al-Zubaidi, S., Yusuf, E. (2020). Environment indoor air quality assessment using fuzzy inference system. ICT Express. 6, 185-194. https://www.sciencedirect.com/science/article/pii/S2405959520301065.
- Eduard, W. 2009. Fungal spores: A critical review of the toxicological and epidemio-logical evidence as a basis for occupational exposure limits setting. Crit. Rev. Toxicol. 39: 799–864.
- Egbuta, M. A., Mwanza, M., & Babalola, O. O. (2017). Health Risks Associated with Exposure to Filamentous Fungi. International journal of environmental research and public health, 14(7), 719. https://doi.org/10.3390/ijerph14070719
- FERREIRA V, SOUSA J: Microbiologia. Lidel, 2000. ISBN 972-757-112-3.

- Forero D. A., González-Giraldo Y., Castro-Vega L. J., & Barreto G. E. (2019). qPCR-based methods for expression analysis of miRNAs. BioTechniques, 67(4), 192–199. https://doi.org/10.2144/btn-2019-0065.
- Fukutomi, Y., & Taniguchi, M. (2015). Sensitization to fungal allergens: Resolved and unresolved issues. Allergology International: official journal of the Japanese Society of Allergology, 64(4), 321–331. https://doi.org/10.1016/j.alit.2015.05.007.
- Huff, W., Kubena, L., Harvey, R., Doerr, J. (1988). Mycotoxin interactions in poultry and swine. J Anim Sci. Sep. 66(9):2351-5.
- Klepsatel, P., Wildridge, D., & Gáliková, M. (2019). Temperature induces changes in Drosophila energy stores. Scientific reports, 9(1), 5239. https://doi.org/10.1038/s41598-019-41754-5.
- Liao, L., Byeon, J. H., & Park, J. H. (2021). Development of a size-selective sampler combined with an adenosine triphosphate bioluminescence assay for the rapid measurement of bioaerosols. Environmental research, 194, 110615. https://doi.org/10.1016/j.envres.2020.110615.
- Haleem, A. M., Hassan, D. M. A., & Al-hiyaly, S. A. K. (2013). Comparative Assessment of Microbial Contamination from Swabs collected within University Facilities. *Journal of Health Science*, 3(2). https://doi.org/10.5923/j.health.20130302.04.
- Maldonado, R. F., Sá-Correia, I., & Valvano, M. A. (2016). Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS microbiology reviews, 40(4), 480–493. https://doi.org/10.1093/femsre/fuw007.
- Millipore (2021). Dichloran glycerol chloramphenicol, Germany, Merck , recuperado em Abril 20, 2021, a partir de https://www.merckmillipore.com/PT/en/product/DG-18-Dichloran-glycerol-chloramphenicol-agar-base,MDA_CHEM-100465#anchor_DS.
- Millipore (2021). Malt Extract Agar, Germany: Merck. Recuperado em abril 20, 2021, a partir de https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Datasheet/1/70145dat.pdf.
- Millipore (2021). Tryptic Soy Agar, Germany, Merck. Recuperado em abril 20, 2021, a partir de https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Datasheet/1/22091dat.pdf.
- Millipore (2021). Violet Red Bile Agar, Germany, Merck. Recuperado em abril 20, 2021, a partir de https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Datasheet/2/40689dat.pdf.

- Mohammadien H. A., Hussein M. T., El-Sokkary R. T. (2013). Effects of exposure to flour dust on respiratory symptoms and pulmonary function of mill workers. Egyptian Journal of Chest Diseases and Tuberculosis, 62, 745–753.
- Moldoveanu, A. M. (2015). Biological Contamination of Air in Indoor Spaces. In: Farhad Nejadkoorki, ed. Current Air Quality Issues. doi:10.5772/58743.
- Nucci, M., & Anaissie, E. (2007). Fusarium infections in immunocompromised patients. Clinical microbiology reviews, 20(4), 695–704. https://doi.org/10.1128/CMR.00014-07.
- Oliveira, J., & Reygaert, W. C. (2020). Gram Negative Bacteria. In StatPearls. StatPearls Publishing.
- Pankaja, S.K., Hu Shib , Keener, K. M., 2018. A review of novel physical and chemical decontamination technologies for aflatoxin in food. Trends in Food Science & Technology. 71, Pages 73-83. https://doi.org/10.1016/j.tifs.2017.11.007.
- Reboux, G., Rocchi, S., Vacheyrou, M., & Millon, L. (2019). Identifying indoor air Penicillium species: a challenge for allergic patients. Journal of medical microbiology, 68(5), 812–821. https://doi.org/10.1099/jmm.0.000960.
- Rintala, H., Pitkäranta, M., Toivola, M., Paulin, L., Nevalainen, A. (2008). Diversity and seasonal dynamics of bacterial community in indoor environment. BMC Microbiology, 8: 56.
- Ross, A. A., Neufeld, J.D. (2015). Microbial biogeography of a university campus. Microbiome, 3:66. doi:10.1186/s40168-015-0135-0.
- Roussel, S., Reboux, G., Bellanger, A. P., Somin, S., Grenouillet, F., Dalphin, J. C., Piarroux, R., & Millon, L. (2008). Characteristics of dwellings contaminated by moulds. Journal of environmental monitoring: JEM, 10(6), 724–729. https://doi.org/10.1039/b718909e.
- Sabino, R., Verissimo, C., Viegas, C., Viegas, S., Brandão, J., Alves-Correia, M., Borrego, L. M., Clemons, K. V., Stevens, D. A., & Richardson, M. (2019). The role of occupational Aspergillus exposure in the development of diseases. Medical mycology, 57(Supplement_2), S195–S205. https://doi.org/10.1093/mmy/myy090.
- Sandoval-Denis, M., Sutton, D. A., Martin-Vicente, A., Cano-Lira, J. F., Wiederhold, N., Guarro, J., & Gené, J. (2015). Cladosporium Species Recovered from Clinical Samples in the United States. Journal of clinical microbiology, 53(9), 2990–3000. https://doi.org/10.1128/JCM.01482-15.

- Sefton A., "The biology of bacteria," Tutorial Topics in Infection for the Combined Infection Training Programme, vol. 1, Oxford University Press, Oxford, UK, 2019.
- Serra R, (2005), Capítulo 1. Micotoxicologia. Braga: Universidade do Minho (file:///C:/Users/user/Desktop/Faculdade/3%C2%BA%20ano/2%C2%BA%20semestre/Est%C3%A1gio/Pesquisas/09-capitulo1-%20Micotoxicologia.pdf).
- Setlow P. 2006. Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. J. Appl. Microbiol. 101:514–525.
- Soppa V. J., Roel P. F. Schins, Frauke Hennig, Bryan Hellack, Ulrich Quass, Heinz Kaminski, Thomas A. J. Kuhlbusch, Barbara Hoffmann, and Gudrun Weinmayr (2014). Respiratory Effects of Fine and Ultrafine Particles from Indoor Sources—A Randomized Sham-Controlled Exposure Study of Healthy Volunteers. 11(7): 6871–6889.
- Sousa, J., Franco, M. H., Rodrigues, M. A., Santos, M., Reis, S. (2001). Riscos dos agentes biológicos. Lisboa: IDICT.
- Stark, P. C., Celedón, J. C., Chew, G. L., Ryan, L. M., Burge, H. A., Muilenberg, M. L., & Gold, D. R. (2005). Fungal levels in the home and allergic rhinitis by 5 years of age. Environmental health perspectives, 113(10), 1405–1409. https://doi.org/10.1289/ehp.7844.
- Stetzenbach, L.D. (2009). Airborne Infectious Microorganisms. Encyclopedia of Microbiology (Third Edition). 2009, Pages 175-182. https://www.sciencedirect.com/science/article/pii/B9780123739445001772#.
- Stryjakowska-Sekulska, M., Piotraszewska-Pajak, A., Szyszka, A., Nowicki, M., Filipiak, M. (2007). Microbiological Quality of Indoor Air in University Rooms. Polish J Env, 16(4):623-632. http://www.pjoes.com/Microbiological-Quality-of-Indoor-Air-r-nin-University-Rooms,88030,0,2.html.
- Tortora, Gerard J.; Berdell R., Funke; Christine L., Case (2009). Microbiology: An Introduction. Berlin: Benjamin Cummings. ISBN 0-321-58420-1.
- Tripathi, N., & Sapra, A. (2020). Gram Staining. In StatPearls. StatPearls Publishing.
- U.S. EPA (2020) Particulate Matter (PM) Pollution. Washington, DC: EPA. Recuperado em Março 23, 2021 (https://www.epa.gov/pm-pollution/particulate-matter-pm-basics).
- Viegas, C., Monteiro A., Araújo Caetano L., Faria T., Carolino E., Viegas S. (2018a). Electrostatic Dust Cloth: A Passive Screening Method to Assess

Occupational Exposure to Organic Dust in Bakeries. Atmosphere; 9(2):64. https://doi.org/10.3390/atmos9020064.

- Viegas C, Twarużek M, Lourenço R, Dias M, Almeida B, Caetano LA, Carolino E, Gomes AQ, Kosicki R, Soszczyńska E, Viegas S. (2020). 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. 11(4):351. https://doi.org/10.3390/atmos11040351.
- Viegas, C., Faria, T., dos Santos, M., Carolino, E., Gomes, A. Q., Sabino, R., & Viegas, S. (2015). Fungal burden in waste industry: an occupational risk to be solved. Environmental Monitoring and Assessment, 187(4). https://doi.org/10.1007/s10661-015-4412-y.
- Viegas, C.; Faria, T.; Monteiro, A.; Caetano, L.A.; Carolino, E.; Gomes, A.Q.; Viegas, S. (2018b). A novel multi-approach protocol for the characterization of occupational exposure to organic dust-Swine production case study. Toxics, 6 (1–14). doi:10.3390/toxics6010005.
- Viegas, C.; Caetano, L.A.; Viegas, S. (2021) Occupational exposure to Aspergillus section Fumigati: Tackling the knowledge gap in Portugal. Environmental Research, 194. doi:10.1016/j.envres.2020.110674.
- WHO (2009). Guidelines for indoor air quality: dampness and mould. Dinamarca: World Health Organization. Disponível em: https://www.who.int/airpollution/guidelines/dampness-mould/en/.
- Ziaee, A., Zia, M., & Goli, M. (2018). Identification of saprophytic and allergenic fungi in indoor and outdoor environments. Environmental monitoring and assessment, 190(10), 574. https://doi.org/10.1007/s10661-018-6952-4.
- Zielińska-Jankiewicz K, Kozajda A, Piotrowska M, Szadkowska-Stańczyk I. (2008). Microbiological contamination with moulds in work environment in libraries and archive storage facilities. Annals of Agricultural and Environmental Medicine; 15:71–78.
- Zulfakar, S. S., Abu, H. M., Abu, B. N. (2019). Microbiological Assessment of Selected Laboratories at a Local University in Malaysia. J Sains Kesihat Malaysia, 17(SI):119-126. doi:10.17576/jskm-2019-14.

13. Apêndices

Apêndice 1: Tabela resumo dos resultados obtidos

Local amostrado	Bactérias totais	Bactérias Gram-	Fungos (MEA)	Fungos (DG18)	Unidade
Armazém Multimédia	4,47E+05	1,00E+03	1,20E+04	9,00E+03	UFC/m²
Auditório	5,17E+05	0,00E+00	1,90E+04	6,00E+03	UFC/m²
Balneário/ Vectiário	5,10E+04	0,00E+00	5,90E+04	6,70E+04	UFC/m²
Bar	5,32E+05	2,00E+03	3,00E+03	9,00E+03	UFC/m²
Bar/Refeitório	5,10E+04	0,00E+00	3,00E+03	1,00E+03	UFC/m²
Biblioteca	6,85E+05	2,00E+03	2,30E+04	1,10E+04	UFC/m²
Casa de Banho	3,20E+04	0,00E+00	3,00E+03	1,00E+03	UFC/m²
Casa de Banho (1º andar)	3,00E+04	0,00E+00	0,00E+00	1,00E+03	UFC/m²
Casa de Banho (2º andar)	7,40E+04	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Contabilidade	1,00E+03	0,00E+00	4,00E+03	8,00E+03	UFC/m²
Contabilidade	1,00E+03	0,00E+00	8,00E+03	6,00E+03	UFC/m²
Contabilidade/ Aprovicionamento	1,00E+03	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Copa	1,91E+05	2,00E+03	4,40E+04	1,80E+04	UFC/m²
Copa (1º andar)	1,45E+04	0,00E+00	9,00E+03	1,00E+03	UFC/m²
Copa (2º andar)	1,45E+04	0,00E+00	6,00E+03	1,00E+03	UFC/m²
Copa 1	1,40E+04	4,00E+03	0,00E+00	2,00E+03	UFC/m²
Copa 2	1,00E+03	0,00E+00	2,00E+03	0,00E+00	UFC/m²
Corredor (impressora)	5,29E+05	1,00E+03	6,00E+03	1,50E+04	UFC/m²
Departamento de Compras (1º andar)	5,82E+05	0,00E+00	1,00E+03	0,00E+00	UFC/m²
Divisão Académica (Atendimento)	7,40E+04	3,00E+03	4,30E+04	7,00E+03	UFC/m²
Entrada	3,80E+05	0,00E+00	1,00E+03	0,00E+00	UFC/m²
Espaço comida de casa	5,52E+05	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Estúdio A1	2,50E+04	0,00E+00	1,00E+03	0,00E+00	UFC/m²
Estúdio Átrio C -1	1,17E+05	3,00E+03	9,00E+03	7,00E+03	UFC/m²
Estúdio D1	2,50E+04	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Gabinete de Comunicação	1,30E+04	0,00E+00	5,70E+04	7,70E+04	UFC/m²

Local amostrado	Bactérias totais	Bactérias Gram-	Fungos (MEA)	Fungos (DG18)	Unidade
Gabinete Docente (1.16)	1,94E+05	0,00E+00	1,00E+03	0,00E+00	UFC/m²
Ginásio	5,10E+04	4,00E+03	4,00E+03	6,00E+03	UFC/m²
Grande Auditório	7,31E+05	0,00E+00	2,40E+04	2,80E+04	UFC/m²
Impressora (1º andar)	1,60E+04	0,00E+00	1,20E+04	1,20E+04	UFC/m²
Impressora (2º andar)	1,30E+04	0,00E+00	2,00E+03	0,00E+00	UFC/m²
Laboratório de Ciências (FQ)	5,10E+04	4,00E+03	3,00E+03	5,00E+03	UFC/m²
Laboratório de Química Inorgânica	1,40E+04	0,00E+00	1,10E+04	1,50E+04	UFC/m²
Laboratório de Química orgânica	3,00E+03	1,50E+04	2,00E+03	1,00E+03	UFC/m²
Logística	1,45E+04	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Oficina	2,00E+04	1,10E+04	1,00E+03	5,00E+03	UFC/m²
Oficina de Cerâmica	5,10E+04	7,00E+03	1,80E+04	1,00E+04	UFC/m²
Pequeno Auditório	3,40E+04	0,00E+00	5,00E+03	1,00E+03	UFC/m²
Pequeno Auditório (Estúdio de Interpretação Cénica)	2,46E+05	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Receção	2,10E+04	0,00E+00	8,00E+03	8,00E+03	UFC/m²
Receção de motoristas	3,70E+04	0,00E+00	1,00E+03	1,00E+03	UFC/m²
Recepção	4,00E+03	0,00E+00	0,00E+00	2,00E+03	UFC/m²
Recursos financeiros	5,06E+05	0,00E+00	4,00E+03	2,00E+03	UFC/m²
Recursos humanos	5,70E+05	0,00E+00	4,00E+03	0,00E+00	UFC/m²
Refeitório	2,30E+04	0,00E+00	2,00E+03	0,00E+00	UFC/m²
Refeitório / Bar	5,00E+03	0,00E+00	2,00E+03	1,00E+03	UFC/m²
Sala de Atendimento	1,00E+03	0,00E+00	0,00E+00	1,00E+03	UFC/m²
Sala de Computadores	6,00E+03	0,00E+00	0,00E+00	1,00E+03	UFC/m²
Sala de Convívio	5,40E+04	0,00E+00	1,00E+03	1,00E+03	UFC/m²
Sala de coro	6,00E+03	0,00E+00	6,80E+04	0,00E+00	UFC/m²
Sala de Dança	4,28E+05	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Sala de Espera/Sala de Aulas	1,00E+03	0,00E+00	1,00E+04	9,00E+03	UFC/m²
Sala de estudo	5,00E+05	2,00E+03	0,00E+00	2,00E+04	UFC/m²
Sala de Música	5,03E+05	1,00E+03	0,00E+00	0,00E+00	UFC/m²
Sala de professores	2,50E+04	0,00E+00	1,40E+04	1,40E+04	UFC/m²

Local amostrado	Bactérias totais	Bactérias Gram-	Fungos (MEA)	Fungos (DG18)	Unidade
Sala de Professores 1	1,30E+04	0,00E+00	1,00E+03	6,00E+03	UFC/m²
Sala de Professores 2	2,00E+03	0,00E+00	1,00E+04	1,61E+05	UFC/m²
Sala de Teatro e Coro	5,10E+04	2,00E+03	0,00E+00	0,00E+00	UFC/m²
Serviços Académicos	5,80E+05	7,00E+03	9,00E+03	9,00E+03	UFC/m²
Serviços Financeiros	1,80E+04	0,00E+00	0,00E+00	0,00E+00	UFC/m²
Total Geral	**9,74E+06**	**7,10E+04**	**6,30E+05**	**6,88E+05**	UFC/m²

Conclusão das avaliações ambiental e laboratorial: Os resultados encontrados evidenciam que os procedimentos e/ou produtos que estão ser utilizados poderão não ser os mais adequados. Futuras avaliações da contaminação microbiológica deverão incidir na avaliação da eficácia dos procedimentos e produtos utilizados nas operações de higienização dos espaços interiores.

Elaborado por:

Raquel Pimenta com o apoio de Sandra Ferreira e Ediane Silva

Aprovado por:

_____