Fungal contamination assessment in Healthcare environments - A bibliographic review

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Abstract

In Healthcare environments fungal presence depend on the medical activities performed, number and types of patients, cleaning frequency and procedures and the type of ventilation systems. The aim of this review article was to identify different methodologies applied to assess fungal contamination in Healthcare environments, as well as to describe the most reported fungi in these environments. This study was based on a systematic search for information and data that have been published in free access sources during the period of 1st January 2000 to 31st December 2020. PRISMA methodology was applied to identify and select studies referring to Healthcare environments where the fungal assessment was performed. The most common Healthcare environments assessed were hospitals (26 out of 56) and the most used sampling methods were active (27 articles). Passive methods were exclusively used in 8 papers, and the combined use of both methods was verified in 21 papers. Concerning analytical procedures, the exclusive use of morphological identification was the most frequent approach (40 out of 56). *Aspergillus* sp., *Cladosporium* sp. and *Penicillium* sp. were the predominant genera found indoors (24 out of 56). There is scientific evidence of fungal contamination present in Healthcare environments. Thus, in order to have an accurate and reliable risk characterization, the combined use of active and passive sampling methods and the use of culture based-methods and molecular tools are of upmost importance.

Keywords: Fungal contamination; Healthcare Environments; Exposure assessment; Active and passive sampling methods; Culture based-methods; Molecular tools

Introduction

The assessment of indoor air quality (IAQ) in Healthcare environments is a critical issue to help to create safe environments and guarantee Public Health. Additionally, the assessment and control of microbial contamination (comprising fungal contamination) in these environments is a prerequisite and vital part of the strategies to prevent and control hospital-acquired infections (Zahar et al., 2017; Viegas et al., 2019b; Ferdyn-Grygierek, 2016).

The distribution and viability of fungal contamination in a given environment is mostly influenced by building characteristics, comprising the availability of water and nutrients for fungal growth and survival, building occupancy, and the outdoor environment (Sudharsanam et al., 2012). In Healthcare environments, fungal presence also depend on the medical activities performed, number and types of patients, cleaning frequency and procedures and the type of ventilation systems (Qudiesat et al., 2009; Jung et al., 2015; Marchand et al., 2016). All these variables determine fungal dissemination and proliferation and influence the indoor sources of contamination in air, water, and surfaces (Sudharsanam et al., 2012).

Most of the reported studies focused the detection of *Aspergillus* species on hospital air, due to its clinical relevance (Sudharsanam et al., 2012). *Aspergillus* is an ubiquitous fungal genus associated with community and hospital-acquired (nosocomial) infections worldwide (Nicolle et al., 2011; Viegas et al., 2019a). More recently, the designations regarding this genus represent sections (or complexes) of closely related species (also mentioned to as cryptic species) that cannot be clearly distinguished morphologically (Lamoth, 2016; Viegas et al., 2019a). *Aspergillus* genus is responsible for more than 80% of pulmonary invasive fungal infections in humans. Additionally, invasive aspergillosis, due to *Aspergillus fumigatus* in 80% of the cases, is the most common invasive fungal infection (Segal, 2009). Several *Aspergillus* sections, such as *Fumigati, Nigri, Aspergilli* and *Nidulantes* were already reported in Healthcare environments (Cabo Verde et al., 2015; Viegas et al., 2019b). Thus, *Aspergillus* should always be evaluated in Healthcare environments assessments and efforts should be implemented to avoid exposure to fungal contamination in highly immunocompromised patients (Patterson et al., 2016).

Concerning the sampling strategy adopted to perform the assessment, it should encompass more than one active method for air sampling together with the use of passive methods, in order to better characterize the risk (Viegas et al., 2019b). The same approach should be followed regarding the assays to be applied. Indeed, combining culture based-methods with molecular tools can overcome each methods limitations unveiling a wider fungal contamination (Viegas et al., 2019b, 2021b, 2020c).

The aim of this review article was to identify different methodologies applied to assess fungal contamination in Healthcare environments, as well as to describe the most reported fungi in these environments. This work is of upmost importance to identify the need of future studies and for the development of effective control measures.

Materials and Methods

This study was based on a well-structured search of published data and information in the public domain between 1st January 2000 and 31st December 2020. Baseline parameters were selected following the PRISMA methodology to identify and select studies referring to health care environments where the fungi assessment was performed, selecting only English and original papers. Appropriate search terms, such as “Healthcare facilities”, “hospital”, “bioaerosols” and “fungi” were employed in the selected databases (PubMed, Scopus and Web of Science. Articles that did not meet the inclusion criteria and duplicates were excluded from further analysis (Table 1). The main findings of the studies were highlighted and compared for consistency, resulting in a final selection of papers (Figure 1). This applied methodology led to 56 papers in all databases (Figure 1).

Table 1- Inclusion and exclusion criteria in the articles selected.

Inclusion criteria	**Exclusion Criteria**
Articles in English language	Articles in other languages
Articles published from January 1st, 2000 to December 31st, 2020	Articles published prior to 2000
Articles related to fungi assessment	Articles related to other microbiologic contaminants
Articles related to environmental samples	Articles related to biological samples
Scientific original articles on the topic/ Journal Articles	Congress abstracts, Reviews, Reports

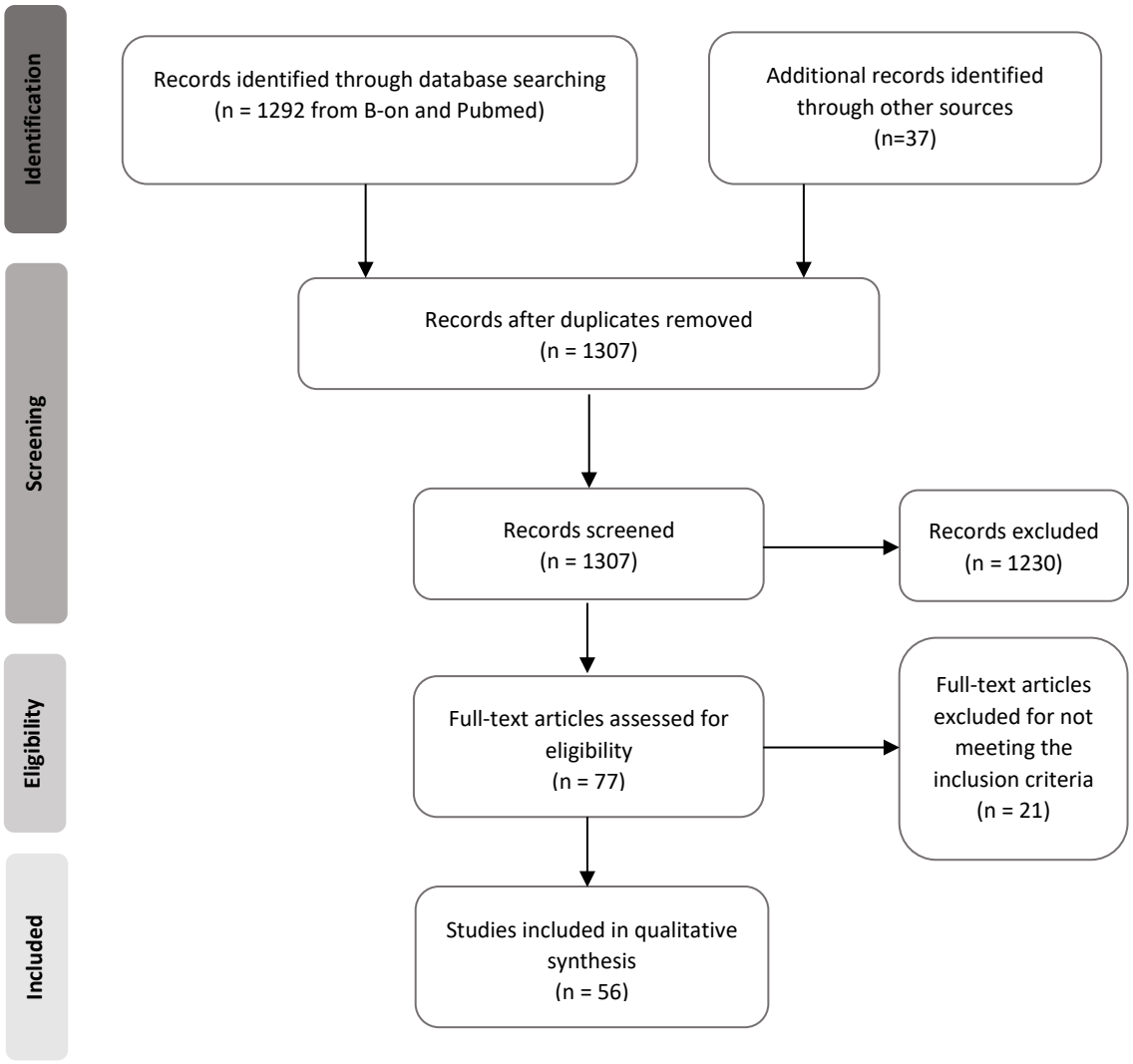

Figure 1 – Article selection based on the PRISMA method

Results

Among the selected studies the most common Healthcare environments assessed were hospitals (26 out of 56), followed by other different environments: 14 teaching hospitals, 8 health care centers, 3 terciary medical centers, 2 podiatry clinics, 1 dental offices, 1 cancer treatment center and 1 private maternity home. In a total of 34 papers only indoor sampling was performed and in a total of 22 studies both indoor and outdoor samples were performed (Table 2).

The most used sampling methods were active (27 articles). Passive methods were exclusively used in 8 papers, and the combined use of both methods was verified in 21 papers. Air sampling through impaction was the most frequently used active sampling, since it was carried out in 18 articles, followed by air impingement (3) air filtration (3), air sampling cassettes (2) and cyclonic air sampler (1). Regarding passive methods, electrostatic dust collectors were the most frequently used, since they were applied in 13 articles in parallel with swabs (8). In addition, settled dust (3), HVAC filters (3) and vacuum cleaner (1) were also applied as passive sampling methods (Table 2).

Concerning analytical procedures, the exclusive use of morphological identification was the most frequent approach (40 out of 56). Additionally, a combination of morphological identification and molecular tools (14 out of 56) and cytotoxicity assessment (2 out of 56 papers) were also verified (Table 2).

Most of the studies reported indoor fungal load lower when compared to the outdoor levels, being verified in 12 out of 56 papers. *Aspergillus* sp., *Cladosporium* sp. and *Penicillium* sp. were the predominant genera found indoors (24 out of 56). Regarding *Aspergillus* sp., few studies performed the identification to the section level (24 out of 56). Additionally, the most prevalent *Aspergillus* sections were *Nigri* (18 out of 24), followed by *Fumigati* (14 out of 24) and *Flavi* (9 out of 24). It was also reported the decrease of fungal contamination after regular disinfection in 2 students health centers and 1 educational hospital (Table 2).

Tabela 2 - Data obtained from the chosen articles.

Database	Title	Country	Health care environment	Sampling methods	Analytical methods	Range/Prevalence	Main findings	References
B-on	Renovation of Contaminated Building Materials at a Facility Serving Pediatric Cancer Outpatients	USA	One facility for pediatric cancer outpatients at a university hospital, indoor and outdoor	Air impaction, Polycarbonate filters for spores, Settle dust samples and Cello-tape samples from the surfaces in the heat pump	Morphological identification	**Air samples:** Outdoor air - Total fungal concentrations ranging from 92 to 1004 CFU/m^3. The most common fungi were *Cladosporium* sp., *Basidiomycetes* sp., and *Penicillium* sp. Indoor air: Total fungal concentrations ranging from 25 to >400 CFU/m^3. The most common fungi were *Cladosporium* sp., *Basidiomycetes* sp., *Penicillium sp.*, *Memnoniella echinata*, *Aspergillus* sp., *Paecilmyces variotii*, *Mucor* sp., sterile fungi. **Bulk material samples:** Microbiological colonization ranged from non-detectable to 14,122,220 CFU/g. *Acremonium* sp., *Stachybotrys chartarum*, *Memnoniella echinata*, *Paecilmyces variotii*, *Aspergillys ustus*, *Rhodatorula*, *Clasdosporium* sp., *Penicillium* sp. and *Aspergillus versicolor* were the most common. ***Dust samples:*** Total concentration *of cu*lturable funi ranged from 2,031,858 to 5,959,084CFU/g. *Cladosporium* sp., *Penicillium* sp., *Rhodoturula*, *Trichoderma koningii*, *Paecilomyces variotti* and *Aspergillus fumigatus* were dominant. **Settled dust samples:** *Aspergillus versicolor*, *Aspergillus sydowii*, *Memmoniella echinata*, *Chaetomium*, *Stachybotrys chartarum* and *Penicillium* were present. **Material surfaces:** *Cladosporium herbarum* and *Cladosporium cladosporioides* were present.	The predominance of one or more species of fungi indoors, that are not present in the outdoor air or in control locations, suggests the presence of an amplifier (growth site) for that species of fungus in the building. Remediation of the contaminated building materials also appears to have contributed to the spread of fungal spores in the return air ceiling plenum. The potential for fungal growth to recur on newly replaced materials is likely, since the leaks in the foundation of the building have not been corrected.	(Weber and Page, 2001)

B-on	Use of surrogate markers of biological agents in air and settled dust samples to evaluate a water-damaged hospital	USA	Two hospital buildings, indoor and outdoor	Air filter cassette, Air impaction, Air sampling cassettes, Vacuumed floor dust collection and vacuumed chair dust collection	Morphological identification	Overal range of culturable fungi in both hospitals and outdoor - **Air measurements**: 6 to 75 CFU/m^3; **Chair measurements**: 3.0 x 10^4 to 1.6 x 10^5 CFU/g; **Floor measurements**: 5.0 x 10^3 to 1.6 x 10^5. The dominant culturable **outdoor** fungal species were *Cladosporium herbarum, Epicoccum* sp.*,* and *Basidiomycetes* sp.*. Alternaria alternata, Aureobasidium pullulans, C. herbarum, Epicoccum nigrum, P. chrysogenum* and *yeasts (*other than *Rhodotorula*) were the predominant fungal species recovered from **chair** and **floor dusts** in both hospitals. *Penicillium* and *Aspergillus* spore counts were higher on the sixth and seventh floors.	In general, indoor concentrations of culturable fungi and fungal spores were lower than outdoors. The results were associated with the building problem.	(Rao et al., 2005)
	Assessment of Bioaerosol Concentrations in Different Indoor Environments	Slovenia	One university faculty, one fast food restaurant, one cultural center, one health centre, one hospital, one meat processing, one mustard processing, one olive processing, and one infant food processing; indoor	Air impaction	Morphological identification	Mean concentration of fungi were 106 CFU/m^3 in health centre and 96 CFU/m^3 in hospital. **Health centre** - *Penicillium* sp., *Cladosporium* sp., *Aspergillus sp.* and *Alternaria* sp. Were the most prevalent; **Hospital** - *Penicillium* sp., *Aspergillus sp.* and *Cladosporium* sp. were the most common.	Indoor air was found to be satisfactory at the health centre and in the hospital.	(Zorman and Jeršek, 2008)

B-on	Study of the Indoor Air Quality in Hospitals in South Chennai, India Microbial Profile	India	Three hospitals, indoor	Settle plate method	Morphological identification	Among yeasts and molds, *Aspergillus niger* and *A. flavus* were commonly isolated and *Candida non-albicans* was also isolated in a few wards.	Use of settle plates for sampling facilitate the monitoring of indoor air quality in hospitals especially those with fewer technical facilities since the method requires no specialist equipment. The mere presence of fungi in hospital air is a concern because many spores can be released leading to an incidence of nosocomial and occupational infections. It was found that the counts were highly influenced by the activity and the ventilation provided.	(Sudharsanam et al., 2008)
	Asthma and respiratory symptoms in hospital workers related to dampness and biological contaminants	USA	Two hospitals, indoor	Air filter cassette, Air impaction, Air sampling cassettes, Vacuumed floor dust and vacuumed chair dust	Morphological identification, molecular identification	Culturable Fungi - **Air measures**: 24 and 10 CFU/m^3 for upper quartile (UQ) and lower quartile (LQ), respectively. **Floor dust measures**: 94,107 and 23,978 CFU/m^3 for UQ and LQ, respectively. **Chair dust measures**: 133,720 and 65,550 CFU/chair for UQ and LQ, respectively.	The levels of fungi in the air showed positive associations with work-related respiratory symptoms, even though the levels of fungi in the air were modest. This data imply new onset of building-related asthma in relation to water damage. The results indicate work related respiratory symptoms in hospital employees were associated with diverse biological exposures including fungi.	(Cox-Ganser et al., 2009)
	Cytotoxicity of *Aspergillus* strains isolated from the neonatal intensive care unit environment	Poland	One hospital (neonatal intensive care unit), indoor	Air impaction, Walls, flor, hand and equipment imprints	Morphological identification and cytotoxicity assessment	**Indoor air**: Mean numbers of fungi varied from 50 to 2370 c.f.u.xm^{-3}. **Imprints**: Mean numbers of fungi varied from 0.04 to 8.83 c.f.u.xcm^{-2}. A majority of the moulds cultured belonged to three genera: *Penicillium* sp., *Aspergillus* sp. *(A. fumigatus, A. ochraceus, A. niger* and *A. glaucus) and Cladosporium* sp.	The numbers of fungi in the morning samples outnumbered those in the evening samples. Moulds were isolated from most of the materials. All of the 17 strains subject to MTT test were cytotoxic. All of the *Aspergillus* strains isolated were cytotoxic.	(Gniadek et al., 2010)
	Improvement of the air quality in student health centers with chlorine dioxide	Taiwan	One student health center, indoor	Air impaction	Morphological identification	The average fungus concentration before disinfection was 802 ± 633 CFU/m^3 and after regular desinfection was 313 ± 117 CFU/m^3.	The number of people present did not increase the fungi concentration. However, the concentration of fungi increased with increased number of persons entering the room per door-opening. The correlation between fungus concentration and humidity and temperature was negative. Regular disinfection is a very effective process.	(Hsu et al., 2010)

B-on	Fungal Microbiota in Air-Conditioning Installed in Both Adult and Neonatal Intensive Treatment Units and Their Impact in Two University Hospitals of the Central Western Region, Mato Grosso, Brazil	Brazil	Two university hospitals, indoor	Air-conditioning unit components swabs	Morpological identification	Fungal concentration was between <100 and 3.56 CFU/g. A concentration of 105 CFU/g was determined in three samples. The most frequently detected fungi were *Aspergillus* sp. (*A. niger, A. paradoxus, A. parasiticus and A. terreus), Penicillium* sp*. (P. expansum, P. chrysogenum, P. griseofulvum, and P. spinulosum), Cladosporium* sp. *(C. cladosporioides, C. elatum), Paecilomyces* sp. *(P. viridis and P. lilacinus), Curvularia geniculat, Cryptococcus* sp*. (C. albidus* and *C. unigutulatus),* and *Rhodotorula mucilaginosa.*	Evaluation of fungal microbiota in the air-conditioning units indirectly determined that the air quality was compromised in both university hospitals analyzed, which constitutes a risk factor for the acquisition of infection in the intensive care units.	(Simões et al., 2011)
	Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres	Italy	One University Hospital, indoor	Settle plate method and Air impaction	Morphological identification	Fungi were isolated only during two separate surgical operations: in the first was identified a colony of *Aspergillus* sp. and in the second was revealed the presence of *Penicillium* sp.	Both methods can be used for general monitoring of air contamination.	(Napoli et al., 2012)
	Application of chlorine dioxide for disinfection of student health centers	Taiwan	One student health center, indoor	Air impaction	Morphological identification	The average fungi concentrations before disinfection were 520 ± 442.4 CFU/m^3. The average residual fungi levels after regular daily interval disinfection were 254.0 ± 43.8 CFU/m^3.	The average fungi concentration before disinfection is less than the maximum permissible level prescribed by the Taiwan EPA. The air quality guidelines prescribed by the Taiwan EPA can best be achieved by applying chlorine dioxide at regular (daily) intervals.	(Hsu et al., 2012)

B-on	Workplace Exposure to Bioaerosols in Podiatry Clinics	Ireland	Fifteen podiatry clinics, indoor	Air impaction and Personal air (sample cassettes)	Morphological identification	The concentrations of fungi ranged from 41.1 to 1914.3 CFU/m^3 and yeasts and moulds ranged from ND to 1028.2 CFU/m^3.	A significant percentage of all the bioaerosols that were in the respirable fraction was representative of yeasts and moulds (65%) and fungi (87%).	(Coggins et al., 2012)
	Assessment of the Levels of Airborne Bacteria, Gram-Negative Bacteria, and Fungi in Hospital Lobbies	Korea	Six hospitals, indoor	Air impaction	Morphological identification	The average levels of fungi were 7.7 × 10 CFU/m^3.	Hospital lobby air was generally contaminated with fungi. Higher levels of fungi were detected after lobby service hours and in the summer. Other significant factors were humidity and air temperature.	(Park et al., 2013)
	Evaluation of total concentration and size distribution of bacterial and fungal aerosol in Healthcare built environments	United Kingdom	One hospital, indoor and outdoor	Air impaction	Morphological identification	The higher concentration of fungi was present **outdoors** (1318 CFU/m^3) and in the **general ward** (1062 CFU/m^3) as compared to the **operation theatres** (22 and 38 CFU/m^3, for conventionally ventilated theatre in the morning and evening, respectively; 5 and 80 CFU/m^3, for laminar flow theatre, in the morning and evening, respectively).	Higher concentrations were found outdoors and in the ward.	(Nasir et al., 2015)
	Microbiological Air Contamination in Premises of the Primary Health-Care	Poland	One primary health-care for adults and children, indoor	Air impaction	Morphological identification	The number of moulds ranged from 15 to 35 CFU/m^3. The predominant of moulds belonging to genera *Aspergillus* sp., *Penicillium* sp. and *Cladosporium* sp.	The highest value of moulds was observed in adult patients' waiting room.	(Karwowska et al., 2013)

B-on	Construction and Application of an Intelligent Air Quality Monitoring System for Healthcare Environment	Taiwan	One public medical center, indoor and outdoor	Air impaction	Morphological identifcation	Total fungi concentrations ranged from 25 CFU/m^3 (**operating suite**) to 269 CFU/m^3 (**outdoor**).	In general, the I/O ratio for total fungi measured from all indoor sampling sites were well below the values indicated in the aforementioned IAQ standards. Total fungi counts were low for restricted sites as compared to public accessible sites. Using CO_2 concentration as a basic indicator of the indoor air quality in a hospital could be possible.	(Yang et al., 2014)
	Identification of airborne microbiota in selected areas in a health-care setting in South Africa	South Africa	One hospital, indoor	Air impaction and Settle plate method	Morphological identification and molecular identification	In the kitchen area and the wards, the fourth sampling round showed higher fungal counts (≤ 4.5 x 10^1 cfu/m^{-3}). *Candida sp., Aureobasidium* sp., *Phoma exigua, Agromyces* sp. and *Penicillium* sp. were the predominant yeasts and moulds identified. Species identification of fungal were *Candida kefyr, Aureobasidium pullulans, Candida krusei, Candida robustad, Candida glabrata, Agromyces rhizospherae, Candida parapsilosis* and *Candida orthopsilosis*.	Fungal genera identified (e.g. *Candida*) also known to cause food spoilage and fungal infections in patients.	(Setlhare et al., 2014)
	Indoor air quality levels in a University Hospital in the Eastern Province of Saudi Arabia	Saudi Arabia	One University hospital, indoor and outdoor	Air impaction	Morphological identification	In **indoor** the total fungal count ranged from 1.68 ± 1.3 CFU/m^3 to 9.6 ± 5.4 CFU/m^3, while in outdoors 8.8 ± 3.9 CFU/m^3. The highest concentrations of different fungal species recorded were *Cladosporium* sp. *and Penicillium* sp*. Yeast, Alternaria* sp*., Mucor* sp*.* and *Aspergillus* sp. were also found in **indoor** and **outdoor**.	The highest and the lowest concentrations of fungi were in emergency room and intensive care unit, respectively. The humidity affected the concentration of airborne fungi.	(El-Sharkawy and Noweir, 2014)
	Microbial contamination of dental unit waterlines and effect on quality of indoor air	Turkey	Twenty dental offices, indoor and outdoor	Water sampling and Air impaction	Morpological identification	**Water samples**: Microfungal counts (MF) in inlet water ranged from 0 to 30 CFU 100 mL^{-1} and in high-speed drills ranged from 0 to 1,600 CFU 100 mL^{-1}. **All water samples**: most prevalent were *Cladosporium* sp., *Aspergillus* sp*., Paecilomyces* sp. *and Penicillium* sp. **Air samples**: The mean of MF counts in indoor air and outdoor air ranged from 5 to 52 CFU/m^3 and from 7 to 154 CFU/m^3, respectively. Indoor air and outdoor air - most prevalent were *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp*.* and *Aspergillus* sp*. Yeast* counts ranged from 3 to 25 CFU/m^3 and *Geotrichum* sp. was identified.	Although low levels of microfungal air (<100 CFU/m3) contamination were detected, potential infections or allergen agents such as *A. alternata*, *C. cladosporioides*, *P. chrysogenum*, *A. niger*, *A. fumigatus*, *A. flavus* and *Paecilomyces* sp. were isolated from water and air samples.	(Kadaifciler and Cotuk, 2014)

B-on	Monitoring of the Environment at the Transplant Unit—Hemato-Oncology Clinic	Czech Republic	One university hospital, indoor	Air impaction, HVAC diffusers swabs and Personal swabs	Morphological identification and molecular identification	All positive culture findings were very low (10 CFU/m^3). Microscopic filamentous fungi were *Aspergillus* sp. *(A. terreus, A. sydowii and A. nidulantes), Trichoderma* sp., *Penicillium sp., Paecilomyces* sp., *Eurotium* sp., and *Monilia* sp.	Microscopic filamentous fungi were cultivated in samples from the sanitary facilities of the patient isolation box and in facilities of the Transplant Unit. There are no representatives of microscopic filamentous fungi in the cleanroom air or on the surfaces of any of the four patient isolation boxes.	(Matoušková and Holy, 2014)
	Fungal Airborne Contamination as a Serious Threat for Respiratory Infection in the Hematology Ward	Iran	One hospital, indoor	Air impaction and Settle plate method	Morphological identification	The mean load of isolated fungi was 10 CFUs/m^3 and 1 CFU/m^2/hour in **air impaction** samples and in **settle plate** samples, respectively. In **air impaction** samples: *Penicillium* sp., *Aspergillus* sp*., Cladosporium* sp*., Yeast*s., *Trichoderma* sp., *Paecilomyces* sp*., Mucor* sp*., Alternaria* sp*., Acremonium* sp*., Fusarium sp.* and *Epicoccum* sp. In **settle plates** samples: *Penicillium* sp*., Aspergillus* sp*., Cladosporium* sp*., Yeasts, Paecilomyces* sp*., Fusarium* sp*., Epicoccum* sp*., Cladosporium* sp., *Alternaria* sp., and *Acremonium* sp..	Active and passive sampling can be used for monitoring the fungal content of air. Installation of special ventilation system equipped with HEPA filters in hematology wards may enhance the quality of air. Observing sanitary protocols for disinfection of the surfaces is imperative for infection control.	(Ghajari et al., 2015)
	Metagenomic Human Repiratory Air in a Hospital environment	China	One hospital, indoor	Air sampling	Morphological identification and molecular identification	*Aspergillus fumigatus* was the most prevalent in most rooms.	Metagenomic technology can easily screen out thousands of microorganisms or cells or genes in a few hours, which is far more rapid than the conventional culture-based method. *Aspergillus fumigatus* was correlated with humidity, but not with visiting people.	(Lai et al., 2015)
	Microbiological assessment of indoor air of teaching hospital wards: a case of Jimma University Specialized Hospital	Ethiopia	One university teaching hospital, indoor	Settle plate method	Morphological identification	The concentrations of fungal aerosols ranged between 2123 to 4168 CFU/m^3.	The concentrations of fungi that were measured in all sampled wards were not significantly different from each other, suggesting that most fungi species present into the air were not human-borne. All wards were heavily contaminated with fungi and this might be potential risk factors for spread of nosocomial infection in this hospital.	(Fekadu and Getachewu, 2015)

B-on	Monitoring of microscopic filamentous fungi in indoor air of transplant unit	Czech Republic	One university hospital, indoor	Air impaction and HVAC diffusers swabs	Morpological identification	**Air samples**: All positive culture findings were very low (10 CFU/m^3). The most frequent established genus was *Aspergillus sp*. (*A. terreus, A. sydowii, A.nidulantes*), *Trichoderma* sp. (*T. harzianum* and *T. itrinoviride*) and *Penicillium chrysogenum, Paecilomyces* sp., *Eurotium amstelodami* and *Chrysonilia sitophila*. **Swabs**: Two positive growth was pesented (0.28%) - *Aspergillus terreus* and *Chrysonilia sitophila*.	Only two cases involved the sanitary facilities of a patient isolation box; the other positive findings were from the facilities of the Transplant unit. The study results demonstrated the efficacy of the HVAC equipment.	(Holý et al., 2015)
	Assessment of multi-contaminant exposure in a cancer treatment center: a 2-year monitoring of molds, mycotoxins, endotoxins, and glucans in bioaerosols	France	One cancer treatment center, indoor	Cyclonic air sampler	Morphological identification and molecular identification	The mean levels of total culturable molds varied from 16.141 to 30.530 CFU/m^3. The major fungal groups identified were *Acremonium* sp., *Cladosporium* sp. (*C. herbarum), Aspergillus* sp. (*A. fumigatus, A. melleus, A.niger, A. sydowii, A.ustus* and *A. nidulantes*) and *Penicillium* sp. *(P. brevicompactum and P. chrysogenum*). Other filamentous fungi identified were *Fusarium culmorum, Mucor* sp., and *Purpureocillium lilacinum*.	110 fungal species were identified. The diversity and complexity of mycoflora in bioaerosol were highlighted. Fungal particle peaks occurred during the summer and autumn. The exposure to airborne mycotoxins was very low and no mutagenic activity was found in bioaerosols.	(Heutte et al., 2017)
	High diversity of airborne fungi in the hospital environment as revealed by meta-sequencing-based microbiome analysis	China	One hospital, indoor	Air sampling pumps with two filters	Morphological identification and molecular identification	The prevalence of *Aspergillus* sp. among fungi was the highest at the species level. *A. fumigatus* was the most prevalent. Among others, *A. niger* and *A. flavus* were also common.	Draft genomes of microorganisms isolated from the hospital environment were obtained by sequence analysis, indicating that investigation into the diversity of airborne fungi may provide reliable results for hospital infection control and surveillance. The potentially harmful airborne pathogens are widespread in the hospital.	(Tong et al., 2017)

B-on	Assessment of bioaerosols in tuberculosis high-risk areas of health care facilities in central Thailand	Thailand	Seven large health care facilities, indoor and outdoor	Air impaction	Morphological identification	The mean indoor and outdoor airborne fungal concentrations were 521.2 and 650.1 CFU/m^3, respectively.	The majority of airborne bioaerosols were in respirable sizes. The I/O ratios were 0.8.The source of airborne fungi was from outdoors. The emergency department had the highest mean indoor and outdoor concentrations of airborne fungi. Potential contributors to the indoor airborne levels are low air change rates, central-type air-conditioning systems, and high relative humidity.	(Sornboot et al., 2018)
	Assessment of Microbial Contamination in Indoor Air of Private Maternity Homes in Moga, Punjab	India	Five private maternity homes, indoor	Settle plate method	Morphological identification	The number of viable fungi were ranging between 79-826 CFU/m^3. The isolated fungi were *Aspergillus* sp*., Mucor* sp*., Rhizopus* sp., *Penicillium sp., Exophiala* sp*.,* and *Absidia* sp.	It was found a high concentration of fungi. The value of fungi load was low in general ward as compare to private wards in the morning and conditions were vice versa in the afternoon. The presence of the family members near the patient were associated with increase of fungi load.	(Kumar et al., 2018)
	A study on microbiological contamination on air quality in hospitals in Egypt	Egypt	Two hospitals, indoor and outdoor	Air impaction, Swabs for sink drain biofilm, Spatula and brush for dust accumulated on floor surfaces and	Morphological identification	Airborne fungi concentrations were in the range of 11 to 566 CFU/m^3 **indoors** and 35 to 664 CFU/m^3 **outdoors**. **Governmental hospital**: *Aspergillus* sp. (*A. flavus*, *A. fumigatus*, A. niger), *Penicillium* sp., Yeasts and sterile hyphae. **Private hospital**: *Aspergillus* sp. (*A. flavus*, *A. fumigatus*, *A. niger*), *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp. and sterile hyphae.	I/O ratios were ≤1 at most sampling sites. The Admission Departments had the worst microbial air quality. Dust was accumulated on air conditioning filters and floor surfaces and these would constitute important sources of airborne fungi inside these hospitals. No clear correlation patterns were obviously found between airborne microorganisms and environmental factors.	(Osman et al., 2018)

B-on	Fungal burden exposure assessment in podiatry clinics from Ireland	Ireland	One hospital based Podiatry outpatient clinic, indoor and outdoor	Air impaction, Surface swabs and Air impingement	Morphological identification and molecular identification	**Indoor**: *Cladosporium* sp., *Aspergillus fumigatus, Stachybotrys chartarum, Rhyzopus* sp. Surfaces: *Cladosporium* sp., *Mucor plumbeus*. **Outdoor**: *Phoma* sp. **Indoor and outdoor**: *Penicillium* sp., *Chrysonilia* sp., *Aureobasidium* sp., *Absidia* sp. and *Chrysosporium* sp. No target fungal species/strains (*Aspergillus* sp. and *Stachybotrys* sp.) were detected by molecular identification for all samples collected. *Trichophyton rubrum* was detected.	These results promote the use of both culture dependent and independent methodologies. All the fungal species identified are considered as indicators of harmful fungal contamination due to their potential health effects. Human activity will contribute to microbial bioburden. Overall, 63.6 % of the evening samples and 46 % of the morning samples surpassed the threshold values (150 CFU/m^3).	(Viegas et al., 2018a)
	Profiles of Environmental Mold: Indoor and Outdoor Air Sampling in a Hematology Hospital in Seoul, South Korea	South Korea	One tertiary medical center, indoor and outdoor	Air impaction	Morphological identification and molecular identification	Fungal concentration in **outside** ranged from 4.2 to 10,112.5 CFU/m^3; inside ranged from 0 to 16.7 CFU/m^3. **Outdoors**: *Aspergillus* sp., *Penicillium* sp. *(P. oxalicum and P. citrinum), Alternaria* sp., *Talaromyces* sp., *Trichoderma* sp., *Paecilomyces* sp., *Byssochlamys* sp., *Curvularia* sp., and *Cercospora* sp. **Indoor**: *Aspergillus* sp., *Penicillium* sp. *(P. hispanicum, P. citrinum and P. chermesinumand) and Alternaria* sp..	Average of fungal concentration in outside was the highest. The outdoor fungal profile was more diverse than the indoor profile. Fungal density affected by relative humidity, presenting higher mean fungal density in summer. *Aspergillus* sp. was dominant outside the hospital in the four seasons; *Alternaria* sp. was dominant in the spring season.	(Cho et al., 2018)
	Assessment of Airborne Bacterial and Fungal Communities in Selected Areas of Teaching Hospital, Kandy, Sri Lanka	Sri Lanka	One teaching hospital, indoor	Settle plate method and Air impaction	Morphological identification and molecular identification	Identification by molecular tools revealed the presence of *Fusarium* sp. and *Aspergillus* sp. The highly pathogenic strains identified by DNA sequencing were *A. nidulantes, A. niger, F. equiseti* and *Trichosporon inkin*.	The hospital air was generally contaminated. All the fungi isolated are identified as highly pathogenic.	(Sivagnanasundaram et al., 2019)
	Assessment of microbiological aerosol concentration in selected Healthcare facilities in southern Poland	Poland	Ten Healthcare facilities indoor	Air impaction	Morphological identification	The concentrations of fungi varied from 1 to 100 CFU/m^3.	The microbial concentrations varied both between the seasons of the year and between the examined facilities. The highest bioaerosol concentrations were observed in most crowded premises.	(Stec and Lenart-Boroń, 2019)

B-on	Electrostatic dust collector: a passive screening method to assess occupational exposure to organic dust in primary health care centers	Portugal	Ten primary health care centers, indoor	EDC sampling	Morphological identification and molecular identification	Fungal contamination levels ranged from 0 to 54,033.97 CFU m^{-2}. *Chrysonilia sitophila, Cladosporium* sp., *Aspergillus* sections *(Versicolores, Nigri, Candidi Fumigati, Circumdati, Aspergilli), Aureobasidium sp., Chrysonilia* sp., *Chrysosporium* sp., *Mucor* sp., *Penicillium* sp., *Stachybotrys chartarum* and *Ulocladium*. Molecular identification revealed no detectable levels of the *Aspergillus* species/complexes targeted (*Fumigati, Flavi, Nidulantes and Circumdati*).	EDC can be applied as a screening method for particle-exposure assessment and as a complementary sampling method for assessing bioburden. The highest fungal count was obtained in the workers 'canteens and in the front offices where patients are first attended, supporting the fact that humans and their activities can be the vehicles for the bioburden within the health care premises.	(Viegas et al., 2019c)
	Phenotypic Detection and Quality Assessment of Indoor Air-Borne Microorganisms Using Passive Air Sampling Technique (Settle Plate) at A Tertiary Care Teaching Hospital in Puducherry	India	One tertiary Care Teaching Hospital, indoor	Settle plate method	Morphological identification	Fungal isolates: *Aspergillus niger, Zygomyces, Fusarium* sp., *Aspergillus flavus* and *Aspergillus fumigatus.*	Most of the identified were a part of the normal aerobic microbial flora; however, a minimum degree of fungal load was observed in the casualty and in the wards due to the constant patient traffic and unrestricted access to patients.	(Valentina and Umadevi, 2019)
	Study on the relationship between the concentration and type of fungal bio-aerosols at indoor and outdoor air in the Children's Medical Center, Tehran, Iran	Iran	One Children's Medical Center, indoor and outdoor	Air impaction	Morphological identification	The average concentrations of total fungal bio-aerosols in the hospital **indoor** and **outdoor air** were 40.48 and 119.6 CFU/m^3, respectively. The most common fungi isolated from the **indoor** were *Penicillium* sp., followed by *Cladosporium* sp., *Aspergillus niger,* sterilized mycelia*, Aspergillus flavus,* Yeast*, Mucor* sp. and *Peacilomyces* sp. The most common fungi isolated from the **outdoor** were *Cladosporium* sp., *Penicillium* sp., *Aspergillus niger* and *Aspergillus flavus*, Sterilized mycelia and *Mucor* sp.	Oncohematology and bone marrow transplantation wards were the most and least contaminated units, respectively. The I/O ratio of fungal aerosols was < 1 at most sampling sites. There was no significant difference between the fungal contamination in the spring and summer.	(Karimpour Roshan et al., 2019)

B-on	Environmental Factors and Ventilation Affect Concentrations of Microorganisms in Hospital Wards of Southern Thailand	Thailand	One hospital, indoor	Biosampler method and settle plate method	Morphological identification	Average fungal concentrations - **Biosampler**: ranged from 134.50 to 487.22 CFU/m^3; **Settle plate**: range from 18.82 to 87.50 CFU/m^3. The overall culturable fungi were *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp*., Curvularia* sp..	The fungal concentration did not exceed the standard limits. The highest concentrations of fungi were measured during and after patient visits and lowest before the visiting time. The number of occupants, temperature and humidity affect fungal concentrations.	(Onmek et al., 2020)
	Exposure assessment in one central hospital: A multi-approach protocol to achieve an accurate risk characterization	Portugal	One hospital, indoor and outdoor	Air impaction, Air impingement, Air filtration, surface swabs, EDC sampling, settled dust and heating collection and HVAC filters collection	Morphological identification and molecular identification	**Morphological identification**: *Penicillium* sp., *Chrysosporium* sp., *Cladosporium* sp., *Aspergillus* sp., *Chrysonilia sitophila*, *Geotrichum* sp., *A. fumigatus*, *Rhizopus* sp., *Aureobasidium* sp., *Chrysonilia* sp., *Scopulariopsis candida*, *A.* sections (*Versicolores*, A*spergilli*, C*andidi*, U*sti*, *Restricti* and C*ircumdati*). **Molecular tools**: *A.* sections *Flavi* and *Fumigati*.	Growth of distinct fungal species was observed on Sabouraud dextrose agar with triazole drugs, such as *Aspergillus* section *Versicolores* on 1 mg/L VORI. Mycotoxins were not detected. 60% of fungal contamination presented I/O > 1. A multi-approach regarding parameters to assess, sampling and analysis methods should be followed.	(Viegas et al., 2020b)

B-on	The Influence of Surgical Staff Behavior on Air Quality in a Conventionally Ventilated Operating Theatre during a Simulated Arthroplasty: A Case Study at the University Hospital of Parma	Italy	One University Hospital, indoor	Air impaction, Settle plate method	Morphological identification	Microbial air contamination values obtained via **active sampling** ranged from 2 ("at rest") to 93 CFU/m^3 ("not correct conditions") and via **passive sampling** ranged from 0 ("at rest") to 16 IMA ("not correct conditions"). No fungi were isolated during "at rest" and "correct condition", while fungi were isolated during "not correct condition" at the operating table (1 CFU/m^3 of *Penicillium* sp.).	The highest levels of microbial and particle contamination, as well as the highest variation in the microclimate parameter, were recorded during the surgical operation where the surgical team behaved "incorrectly". The microbial contamination levels were below the recommended values.	(Pasquarella et al., 2020)
Pubmed	Bioaerosol characteristics in hospital clean rooms	Taiwan	One hospital, indoor	Air impaction	Morphological identification	Fungal concentrations - **Intensive care units**: 0 to 319 CFU/m^3; **Bone marrow transplant unit**: no fungal were detected; **Operating room**: 0 to 51 CFU/m^3. *Penicillium* sp. and yeast were the most predominant.	Regarding bioaerosol characteristics, the fungal concentrations varied over a wide range, but very few microorganism colonies were observed in class 100 clean rooms.	(Li and Hou, 2003)
	Risk of bioaerosol contamination with *Aspergillus* species before and after cleaning in rooms filtered with high-efficiency particulate air filters that house patients with hematologic malignancy	USA	One cancer research hospital, indoor and outdoor	Surface swabs and air impaction	Morphological identification	Positive for *Aspergillus* species - **Surface samples**: 1.6%; **Bioaerosol samples**: 60.2% and 7.3% for outdoor and indoor, respectively. Geometric mean density of colonies - **Room**: 3.0 CFU/m^3, **Shower**: 8.0 CFU/m^3, Toilet: 45.5 CFU/m^3. *A. niger* was the most commonly detected species indoors and outdoors; less commonly isolated were *A. flavus* and *A. tamari*.	Bioaerosol samples collected in the afternoon were more likely to contain *Aspergillus* species than were those collected in the morning. The time frame for detection of *Aspergillus* species may be related to patient and visitor traffic. Concentration of bioaerosol contamination with *Aspergillus* species was increased in rooms sampled 1 hour after cleaning compared with rooms sampled before cleaning, suggesting a possible correlation between reentrained bioaerosols after cleaning and the risk of nosocomial invasive aspergillosis.	(Lee et al., 2007)

Pubmed	A study of air microbe levels in different areas of a hospital	Spain	One hospital, indoor	Air impaction	Morphological identification	Fungal load in operating theatres (OT) ranged from <0.03 to 7.33 CFU/m^3; in maternity ward (MW) ranged from 0.03 to 44.67 CFU/m^3; in hospital rooms ranged from 0.03 to 266 cfu/m^3. Mean *Aspergillus* concentration were <0.03, 0.10 and 0.15 in OT, MW and rooms, respectively. In OT, 10.3% of the samples revealed presence of fungi with 1.42% representing *Aspergillus* sp. (*A. fumigatus* and *A. flavus*).	Low levels of airborne fungi can be reached in clean rooms. Seasonal changes were not detected in fungal levels at any of the sites. There were an increase in fungal levels (more than 100 CFU/m^3), particularly *Aspergillus* sp., after renovation.	(Ortiz et al., 2009)
	Distribution characteristics of airborne bacteria and fungi in the general hospitals of Korea	South Korea	Five hospitals, indoor and outdoor	Air impaction	Morphological identification	Airborne fungi levels ranged from 65 to 156 CFU/m^3. The predominant genera of airborne fungi identified were *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., and *Alternaria* sp.	In airborne fungi, main lobby showed the highest level. Indoor concentration of airborne fungi turned out to be higher than the outdoor concentration. The four predominant airborne fungi usually had the highest identification rate on stage 1 (>7.0 µm).	(Kim et al., 2010)
	Characterization of indoor bioaerosols from a hospital ward in a tropical setting	India	Tertiary Healthcare facility, indoor and oudoor	Settle plate method, Air impingement and Filtration (personal sampling filter cassette loaded with gelatin filter)	Morphological identification	Concentrations of fungi - **Settle plate**: 0 to 13 CFU/plate; **Air Impingement**: 0 to 3.547E+03 CFU/m^3; **Filtration**: 0 to 1.515E+04 CFU/m^3. Most common microorganisms isolated - **Settle plate**: *Aspergillus* sp. (*A. niger*, *A. fumigatus* and *A. flavus*) and *Absidia* sp. **Impingement**: *A. niger, A. fumigatus* and *A. terreus*. **Filtration**: *A. niger* and *A. fumigatus*. Microbial loads in indoor air were less than the outdoor air.	There was no significant temporal variation in airborne microbial loads irrespective of methods. It is evident that in the context of a tropical setting, where hot and humid conditions prevail throughout the year irrespective of the prevailing seasons, changing climatic conditions may not have an impact on the airborne microbial concentrations. Settle plate method was found to capture microorganisms efficiently with little variation in duplicate samples.	(Sudharsanam et al., 2012)

Pubmed	Hospital environment and invasive aspergillosis in patients with hematologic malignancy	USA	One hospital, indoor	Air impaction surface samples and water samples	Morphological identification	*Aspergillus* sp. was isolated from 21 surface samples and 46 bioaerosol samples. *Aspergillus* sp. was not isolated from any water samples. The majority (90%) of the positive bioaerosol samples had ≤ 10 CFU/m3 of *Aspergillus* sp.	Only 2 patients developed nosocomial invasive aspergillosis. No correlations were found between *Aspergillus* species isolated from the hospital rooms and those causing invasive aspergillosis.	(Lee et al., 2012)
	Post-flood measurement of fungal bio-aerosol in a resource-limited hospital: can the settle plate method be used?	Thailand	One tertiary-care hospital, indoor	Air impaction and settle plate method	Morphological identification	In open-ventilation units, measured fungal bioaerosols ranged from 60 to 3030 CFU/m^3; in closed-ventilation units, ranged from 0 to 1980 CFU/m^3.	There was a positive correlation between the two methods only in the open-ventilation units. air sampling are limited. Settle plates can be used as an alternative to air impaction in open-ventilation hospital units where the pattern of air flow facilitates deposition of mould spores.	(Khawcharoenporn et al., 2013)
	Improving the indoor air quality of respiratory type of medical facility by zeolite filtering	Taiwan	One hospital,, indoor and outdoor	Air impaction	Morphological identification	The highest concentration of fungi was found in the outdoor environment in the afternoon (≈1600 CFU/m^3); and the lowest in one ward in the morning (≈0 CFU/m^3). The cumulative bio-aerosol removal after AgZ filtering for fungi were 1088 CFU g^{-1} after 24 hr and 2,700 CFU g^{-1} after 120 hr.	The results indicated that AgZ filtering could continue effectively reduce fungi concentrations to below EPA regulation.	(Shen et al., 2014)
	Aerosol distribution during open suctioning and long-term surveillance of air quality in a respiratory care center within a medical center	Taiwan	One respiratory care center within a medical center (one hospital), indoor	Air impaction	Morphological identification	The fungal concentration averaged 36.7 CFU/m^3 over the whole year. The highest airborne fungal were 122.37 CFU/m^3 and the lowest were 7.92 CFU/m^3. The predominant fungal genera included *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., Yeast*, Scopulariopsis* sp., *Verticillium* sp., *Trichoderma* sp., *Paecelomyces* sp., *Fusarium* sp. *and Acremonium* sp..	The highest concentrations of fungi were in July afternoon and the lowest in February afternoon.	(Chung et al., 2015)

Pubmed	Microbiological assessment of indoor air quality at different hospital sites	Portugal	One hospital, indoor and outdoor	Air impaction	Morphological identification	**Indoor air fungal concentration** - Emergency service (ES): 27 to 933 CFU/m^3; Surgical ward (SW): 1 to 32 CFU/m^3; Operating theatre (OT): below detection limit of 1 CFU/m^3. *Penicillium* sp., *Aspergillus* sp. (*A. fumigatus, A. nidulantes, A. glaucus and A. niger*), *Cladosporium* sp., *Chrysonilia* sp., *Chrysosporium* sp., *Scopulariopsis brevicaulis*. Regarding the fungal population, the results revealed a reduction from outdoors to indoors.	In the SW there existed a site with fungal indoor concentrations higher than those detected outdoors. In the ES, quantitative values of fungi were found to be significantly higher than outdoors, suggesting fungal contamination sources from within and/or a concentration effect of fungi from outside to indoors. Significant differences were detected between summer and winter fungal air loads of ES and SW. The results indicated the efficiency of hospital ventilation/filtration systems in reducing indoor air fungal loads.	(Cabo Verde et al., 2015)
	Concentration and type of bioaerosols before and after conventional disinfection and sterilization procedures inside hospital operating rooms	Iran	One educational hospital, indoor	Air impaction	Morphological identification	The concentrations of fungi before cleaning procedures were limited from 4.83 to 18.40 CFU/m^3 and after cleaning procedures ranged from 1.90 to 8.90 CFU/m^3. The main fungal species identified in the indoor (before vs. after sterilization) were *A. fumigatus* (25.6 vs. 18.3%), *A. niger* (11.6 vs. 5.8%), *Penicillium* sp. (5.5 vs. 3.3%), *Alternaria* sp. (2.8 vs. 0.7%), Fusarium sp. (9.7 vs. 3.7%), *Mucor* sp. (15 vs. 12.7%), *Cephalotrichum* sp. (7.9 vs. 0.8%), *A. flavus* (24.6 vs. 18.5%), *Cladosporium* sp. (2.6 vs. 1.6%), and *Trichoderma* sp. (0.9 vs.0%).	The mean concentrations of airborne fungi before and after disinfection and sterilization were significantly lower compared to the suggested value. Temperature and humidity had significant relationships with the concentration of airborne fungi after disinfection and sterilization.	(Dehghani et al., 2018)
	Microbiological analysis of bioaerosols collected from Hospital Emergency Departments and ambulances.	Poland	Indoor air: Ten health emergency departments, Nine ambulances, and nine offices; outdoor air	Air filtration sampling (personal Button Sampler)	Morphological identification	**Indoor air** concentrations of fungi - Health Emergency Departments: 3.4 × 100 to 8.1 × 10^1 CFU/m^3; Ambulances: 6.7 × 100 to 6.5 × 10^2 CFU/m^3; offices: 0 to 7.9 × 10^2 CFU/m^3. **Outdoor air** concentrations of fungi: 1.5 × 10^2 to 8.2 × 10^2 CFU/m^3. The prevalent fungi species belonged to the genus *Aspergillus* sp. (indoor: *A. flavus* and *A. fumigatus* were prevalent) and *Penicillium* sp. Ambulances: *Penicillium glabrum* (Wehmer) Westling, Bjerkandera adusta (Willd.), and Engyodontium album (Limber) were also prevalent. In **outdoor**, species were similar, except for *A. flavus* that was not detected.	The quantitative assessment of examined indoor air was similar to control outdoor air and was relatively low. The level of fungi did not exceed the recommended level in public spaces.	(Bielawska-Drózd et al., 2018)

Pubmed	Investigating the effect of several factors on concentrations of bioaerosols in a well-ventilated hospital environment	Iran	One hospital, indoor	Settle plate method	Morphological identification	Only 6 fungi were found in all of the samples.	All the wards are below the recommended levels. The bioaerosol concentrations was not affected significantly by the temperature, relative humidity, working shift, season, number of people and various size fractions of particulate matter (except for > 10 µm).	(Mousavi et al., 2019)
	The effect of temperature on airborne filamentous fungi in the indoor and outdoor space of a hospital	Iran	One educational hospital, indoor and outdoor	Air impaction	Morphological identification	**Indoor**: The mean of airborne fungal density was 90, 113, and 24 CFU/m^3 at 15, 25, and 37 °C, respectively. The predominant fungi were *Fusarium* sp*., Penicillium* sp., *Paecilomyces* sp., and *Aspergillus niger*. **Outdoor**: The density of fungi in outdoor air was more than indoor spaces. The predominant species was *Fusarium* sp..	In indoor, the highest density was detected in emergency room and the lowest of them was in neonatal intensive care unit and operating room. The distribution of indoor predominant airborne microorganisms was similar to the outdoor space. The airborne fungi transmit from outdoor to indoor space by ventilation, door, and window. The incubation temperature had effect on airborne fungi remarkably.	(Abbasi and Samaei, 2019)
Others	*Aspergillus* spp. prevalence in Primary Health Care Centres: Assessment by a novel multi-approach sampling protocol	Portugal	Ten primary health care centers, indoor and outdoor	Air impaction, Air impingement, Surface swabs, EDCs, settled dust, and HVAC filters	Morphological identification and molecular identification	Overall*, Aspergillus* section *Nigri* was the most prevalent. Sections *Nidulantes, Aspergilli, Candidi, Flavi, Fumigati* and *Circumdati* were also found.	*Aspergillus* sp. was observed in all PHCC, with highest prevalence on floor surface swabs. The use of a multi-approach sampling protocol to assess *Aspergillus* burden in the analysed PHCC has greatly contributed to risk characterization.	(Viegas et al., 2019a)

Others	Bioburden in Healthcare centers: Is the compliance with Portuguese legislation enough to prevent and control infection?	Portugal	Ten primary health care centers, indoor and outdoor	Air impaction, Air impingement, Surface swabs and HVAC filters	Morphological identification and molecular identification	Fungal contamination - **Indoor air**: 4 to 1.104 x 10^3 CFU/m^3; Surfaces: 0 to 2.2 x 10^5 CFU/m^3; HVAC filters: 0 to 205.5 x 10^3 CFU/m^3. **Air samples:** *Chrysonilia sitophila, Cladosporium* sp., *Penicillium* sp. **Surface samples:** *Phoma sp., Cladosporium* sp., *Aspergillus* sp., *Penicillium* sp., *Chrysosporium* sp. HVAC filters: *Penicillium* sp. ***Aspergillus* sp.*:* Air samples:** *Aspergillus* sections *Fumigati, Circumdati, Nigri, Nidulantes* and *Aspergilli;* **Surface samples:** *Aspergillus* sections *Nigri, Fumigati, Circumdati, Nidulantes;* **HVAC filters:** *Aspergillus* sections *Nigri, Nidulantes, Circumdati, Aspergilli* and *Flavi*.	Regarding fungal load, 60% did not comply with the quantitative guideline (I/O < 1). The presence of mycotoxins was found both in air and HVAC filter samples. Positive fungal growth in at least one azole supplemented media was observed. The Portuguese legislation is not enough to ensure IAQ in health care settings. The multi-approach sampling protocol used in this study allowed to unveil a more real scenario regarding exposure to bioburden.	(Viegas et al., 2019b)
	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?	Portugal	One hospital (Clinical Pathology Service), indoor	Ventilations grids swabs and EDC sampling	Morphological identification, molecular identification, and cytotoxicity assesment	**Mean fungal contamination** - Swabs: 2.4 x 10^2 (MEA) and 0.6 x 10^2 (DG18); EDC: 0.7 x 10^2 (MEA) and 1.7 x 10^2 (DG18). **Fungi prevalence** - **Swabs**: *Chrysonilia sitophila, Penicillium* sp*. and Cladosporium* sp.*;* **EDCs:** *Cladosporium* sp.*, C. sitophila and Mucor* sp*.* ***Aspergillus* sp.** - **Swabs**: Sections *Nigri, Circumdati* and *Versicolores* were found in both media, and *Aspergilli* was only found in DG18; **EDC:** *Nidulantes* in both media, and *Circumdati* in DG18. **Molecular tools**: Sections *Fumigati and Nidulantes*.	Fungal growth on azole-supplemented media was observed in eight EDC samples. No mycotoxins were detected in any of the samples. Swabs and EDCs unveiled a more complete characterization of the bioburden. Culture-based method and molecular tools used in parallel should be used.	(Viegas et al., 2020d)
	Settled dust assessment in clinical environment: useful for the evaluation of a wider bioburden spectrum	Portugal	Ten primary health care centers, indoor	Settled dust	Morphological identification and molecular identification	Fungal load: ranged from 0 CFU.g^{-1} to uncountable. *Chrysonilia sitophila, Penicillium* sp., *Cladosporium* sp*.* were prevalent*. Aspergillus* sections found: *Nigri, Fumigati, Nidulantes* and *Candidi.*	Fungal growth was observed in the different antifungal-supplemented media. Three out of 10 settled dust samples were contaminated by mycotoxins. Settling dust sampling in a routine way might provide useful information about bioburden exposure.	(Viegas et al., 2020b)

Discussion

It is well known that Healthcare environments are enclosed indoors spaces with a high risk of cross-infection between patients and workers. Studies in European hospitals (Kaoutar et al., 2004; Viegas et al., 2020b) indicated that nosocomial infections contribute substantially to morbidity and mortality rates and that many of these infections are spread by airborne pathogens (Kowalski, 2017; Viegas et al., 2020c). Several day-to-day operations from Healthcare facilities potentiate bioaerosols dispersion (Gupta et al., 2009, 2010; Tang et al., 2012, 2013; Viegas et al., 2020c), contributing to infectious diseases transmission (Viegas et al., 2020a).

Most of the analysed articles (27 out of 56) collected air samples as sampling approach, being impaction the most common sampling method. Impaction samplers gather particles from air with a particular flow rate and position them on a collection surface, such as petri dishes containing agar medium (Beard et al., 1980). As this method relies solely on culture-based the aim of their use should be to assess viability of microorganisms and their inflammatory and/or cytotoxic potential (Croston et al., 2016). Indeed, only with information regarding viable part of the fungal contamination it is possible to characterize the inflammatory capacity that varies depending on the microbial composition of the bioaerosol (Timm et al., 2009; Dias and Viegas, 2020). Nonetheless, it is important to point out that this sampling process has certain drawbacks, as it only evaluates species that are cultivable, underestimating the total number of microorganisms in samples at non-culturable state, but which can still cause illness (Cox et al., 2020). Another limitation is that, in indoor environments, the air is not uniform in space or time, it is often influenced by the type of operation (Stellman, 1998) and its speed, and the high velocity of the air flow, can result in the loss of some particles (Beard et al., 1980). Therefore, it is important to ponder the use of other active sampling methods and passive methods to complement the impaction method. As previously reported, some studies (21 out of 56) combined both sampling methods improving the accuracy of the assessment (Viegas et al., 2016; Dias and Viegas, 2020).

Regarding assays used to characterize fungal contamination the exclusive use of morphological identification was the most frequent approach (40 out of 56). However, it is important to highlight that the use of culture-based methods to preform fungal morphological identification has its own disadvantages like how the growth rate and requirements of various fungal species influence other species in mixed cultures. For example, the rapid growth of a particular species may induce overgrowth and, ultimately, chemical rivalry, which may hinder the growth of other species (Viegas et al., 2015) which leads to a loss of accuracy in the outcome of the analysis. Those drawbacks are important to support the idea of combining culture-based methods and molecular tools as seen in 14 out of the 56 papers included in this study. Molecular tools have certain characteristics that allow them to be more effective 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, as well as toxigenic strains of some fungal species (Amann et al., 1995; MacNeil et al., 1995; Viegas et al., 2015). The complement between culture-based methods and molecular tools reinforce the recommendation to be used in parallel.

Aspergillus sp., *Cladosporium* sp. and *Penicillium* sp. were the most reported to be the predominant genus found indoors. A rise of infections induced by the opportunistic *Aspergillus* sp. in immunocompromised patients has been identified, as well as an increase in antifungal resistance, both in clinical and in the environment (Fairlamb et al., 2016; Nature microbiology, 2017; Viegas et al., 2020c). Among *Aspergillus* sections the most reported were *Nigri*, *Fumigati* and *Flavi* and all present toxigenic potential (Varga et al., 2015; Viegas et al., 2018b, 2020b) which represents an increased occupational risk, since due to their presence exposure to

mycotoxins should be also considered (Lamoth, 2016; Dias and Viegas, 2020). Therefore, it is crucial to standardize a broader and more rigorous assessment of *Aspergillus* contamination to ensure an accurate exposure assessment and an effective surveillance of the *Aspergillus* occupational health threat (Viegas et al., 2020a).

Conclusions

There is scientific evidence of fungal contamination present in Healthcare environments. Thus, in order to have an accurate and reliable risk characterization, the combined use of active and passive sampling methods and the use of culture based-methods and molecular tools are of upmost importance.

Acknowledgments

H&TRC authors gratefully acknowledge the FCT/MCTES national support through the UIDB/05608/2020 and UIDP/05608/2020.

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