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The Influence of Carpeting, Human Activity and Number of Beds on Airborne Fungi Concentration in Hotel Bedrooms

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Featured Application: The results of the present work can be applied in the design of more effective (regarding both health and financial costs) sanitation protocols for indoor areas.

Abstract: In urban environments, people spend about 90% of their time indoors, where strong indicators of air-borne contaminants have been found. Currently, there are no reports on the fungal presence and distribution in the air of hotel bedrooms. In this study, we assessed the presence of airborne fungi in bedrooms from three hotels and correlated with room characteristics. We sampled 100 L (L) of air from hotels in Nuevo León, Mexico, then fungi colony forming units (CFU) were measured and identification was made based on morphological features. Variables considered were the presence of carpet, number of beds, cleaning status for the room and floor number. *Penicillium*, *Cladosporium* and *Aspergillus* exhibited the highest CFU concentration and frequency. A slight tendency was observed towards lower fungi concentrations when rooms had been cleaned before sampling. Statistical differences were found between rooms with carpet vs. no carpet, and one vs. two beds. Furthermore, a correlation between floor number and fungi concentration was observed with correspondence to the hotels' room assignment protocol. These findings offer new variables to take into consideration when designing and implementing preventive or corrective sanitization procedures to improve their efficiency and could be relevant for hotel bedrooms as well as any other type of room.

Keywords: indoor air quality; fungal spores; hotel bedrooms; CFU concentration

1. Introduction

Indoor air quality is especially important for human health since people spend about 90% of their time indoors in modern urban environments. Public health scientists have found that closed spaces may be heavily charged with air-borne contaminants [1,2].

Bioaerosols are a main component of indoor airborne contaminants, which comprise microorganisms and can be generated from various natural and anthropogenic sources. Microbes can grow on a variety of items and due to their small size and mass, they get easily transported, persisting in the air for a long time. Sources of indoor bioaerosols include outdoor air, building materials, furnishings, human occupants, animals, plants and organic waste [3–6].

Indoor fungi are common and important allergens, which also represent a potential risk of infection, both superficial and invasive, especially in immunocompromised individuals, and mycotoxin production is another contributory factor to the health risks posed by the fungal species belonging to this family [7]. A great amount of information has been published about indoor fungi, however, there are currently no official standards to regulate the presence of fungi on indoor air, mostly due to the lack of correlation between precise levels of fungal concentrations and health effects. In addition, there is no gold standard method to identify and quantify airborne fungus [3,8,9].

Few studies have been conducted in Mexico concerning the quantification of indoor fungi. In 2011 Ponce-Caballero et al., collected air samples from thirty domestic homes houses in Merida, Yucatan, Mexico, found the presence of the genera *Cladosporium*, *Fusarium*, *Acremonium* and *Alternaria* in nearly 50% of the samples [10]. Another study was conducted in a hospital from Xalapa City in Mexico, in which different surfaces were sampled, showing the predominant presence of genera from *Cladosporium*, *Microsporum*, *Aspergillus* and *Penicillium*; they concluded that all areas of the hospital have pathogenic fungi and these were collected from surfaces, not air, highlighting the lack of information regarding air fungi in our country [11]. In the present study, we evaluated for the first time, three hotels in Nuevo León, Mexico, aiming for a better understanding of the indoor fungal presence in this type of building and generating advice for each hotel's staff afterwards, both related to the customers safety and the health of the workers.

2. Materials and Methods

2.1. Area of Study

Three hotels located at Monterrey, Mexico were evaluated for their indoor air fungal concentrations in guest rooms only. For confidentiality purposes, they are referred to as hotel 1, 2 and 3; their characteristics are shown in Table 1.

Table 1. General characteristics of hotels included in this study.

Hotel	No. of Floors	No. of Bedrooms	Rating (stars)	Location
1	9	199	3	Downtown Monterrey
2	17	191	4	Downtown Monterrey
3	4	160	4	15km south of downtown, Monterrey

For hotels 1, 2 and 3, 80, 84, and 35 rooms were samples, respectively. Variables included in this study were the presence of carpet, number of beds (1 vs. 2), housekeeping status of the room at the time of sampling (cleaned vs. not cleaned) and floor number of the room. Air conditioning was present in all of the rooms without any other ventilation mechanism (e.g., ceiling fans, opening windows) so this was not considered as a variable. The aim was to find possible correlations between these variables and the concentration of fungal propagules in air, to discuss causality.

2.2. Sampling

Air samples were taken using an AirTest® device (LCB food safety, Boz, France). Following a previous report [8] a total of 100 L of air was impacted on Petri dishes containing Rose bengal-malt extract-agar (RBME; BD, USA). The sampling device was located at 1.5 m above the ground and all samples were replicated 2 times. All samples were taken during the same season and between 14:00 and 18:00 h.

2.3. Microbiological Analysis

From day 5 of incubation, CFU were quantified, and microscopic structures were identified based on morphological features including colony features (color, size, shape and hyphae), and microscopic characteristics (hyphal diameter, presence of septa, conidial size,

shape and disposition, etc.) [12–14]. Non sporulating fungi after 30 days were reported as sterile mycelium.

2.4. Statistical Analysis

Wilcoxon signed-rank test was applied because fungi CFU values were not normally distributed. Linear regression was performed to evaluate the positive or negative relationship among variables, prior to such analysis a $\log_{10}$ transformation was performed on the fungi count to normalize the values. Shapiro–Wilk normality was performed for validating the normal distribution property of the variable. As a final step, a Pearson correlation matrix test was performed on the variables to validate the results of the linear regression. A p-value less than 0.05 was considered statistically significant.

3. Results

Table 2 shows the global results regarding fungal concentration; the highest fungal CFU counts were observed in hotel 1, while hotel 3 showed the lowest. *Penicillium* exhibited both the highest CFU concentration and frequency (i.e., presence in air independently of concentration) in hotels 1 and 2, while for hotel 3 *Cladosporium* notoriously exhibited the highest concentration but *Aspergillus* was the most frequently isolated genus (Table 3).

Table 2. Total CFU count (per m^3 of air) by genus and their proportional concentration.

Fungi	CFU Count (%)		
	Hotel 1	Hotel 2	Hotel 3
Penicillium sp.	1,817 (40.3)	718 (31.1)	464 (3.0)
Cladosporium sp.	773 (17.1)	626 (27.1)	11,017 (70.8)
Neoscytalidium sp.	645 (14.3)	333 (14.4)	
Aspergillus sp.	560 (12.4)	118 (5.1)	3065 (19.7)
Fusarium sp.	275 (6.1)	241 (10.4)	
Sterile mycelium	169 (3.7)	108 (4.7)	221 (1.4)
Yeasts	113 (2.5)	125 (5.4)	556 (3.6)
Colletotrichum sp.	55 (1.2)	16 (0.7)	
Rhizopus sp.	42 (0.9)	2 (0.1)	
Alternaria sp.	32 (0.7)	11 (0.5)	210 (1.4)
Paecilomyces sp.	21 (0.5)	1 (<0.1)	10 (0.1)
Syncephalastrum sp.	5 (0.1)		
Geotrichum sp.	1 (<0.1)	2 (0.1)	
Curvularia sp.		2 (0.1)	
Trichoderma sp.	1 (<0.1)	4 (0.2)	
Bipolaris sp.	2 (<0.1)		10 (0.1)
Ulocladium sp.	1 (<0.1)	1 (<0.1)	
Scopulariopsis sp.	2 (<0.1)		
Nodulisporium sp.		1 (<0.1)	

Table 3. Frequencies of fungi regardless of their concentration (Number of rooms where fungi appeared/total of rooms).

Fungi	Hotel 1	Hotel 2	Hotel 3
Penicillium sp.	0.95	0.912	0.429
Aspergillus sp.	0.938	0.713	0.829
Cladosporium sp.	0.725	0.812	0.771
Neoscytalidium sp.	0.613	0.525	
Sterile mycelium	0.75	0.663	0.286
Fusarium sp.	0.5	0.525	
Yeasts	0.288	0.275	0.543
Alternaria sp.	0.188	0.125	0.343
Colletotrichum sp.	0.15	0.175	
Paecilomyces sp.	0.138	0.025	0.029

Table 3. *Cont.*

Fungi	Hotel 1	Hotel 2	Hotel 3
Syncephalastrum sp.	0.062		
Rhizopus sp.	0.038	0.025	
Trichoderma sp.	0.038	0.05	
Bipolaris sp.	0.025		0.029
Geotrichum sp.	0.013	0.013	
Ulocladium sp.	0.013	0.013	
Scopulariopsis sp.	0.013		
Curvularia sp.		0.025	
Nodulisporium sp.		0.013	

Statistical differences were found when data were grouped according to the presence of carpet and the number of beds, with higher fungi concentration when carpet was present and when the room had two beds (Figure 1A,C). Regarding the housekeeping status of the room, there was a slight tendency towards lower concentrations of fungi in air when rooms had been already cleaned at the time of sampling, but no statistical difference was found (Figure 1B). According to Wilcoxon signed-rank test, linear regression considering both conditions explains 29% of the variability. Rooms with one vs. two beds seemed to increase 0.2 log10 units of fungi count and the presence of carpet vs. no carpet in the room present an increase of 0.6 log10 units of fungi count. Pearson correlation matrix showed a medium strength of association ($r = 0.31$) among the number of beds and fungi count, and a large strength of association ($r = 0.51$) among carpet and no-carpet rooms and fungi count. In both cases there is a positive correlation, which provides a validation with what was shown in the linear regression analysis.

Is it noteworthy that the fungal proportion of fungal genera changed noticeably when data was grouped according to the analyzed variables (Figure 2). It is even more interesting that, for variables where a significant difference was found (i.e., presence of carpet and number of beds), *Penicillium* is the most abundant genus in the higher spore concentration condition. The explanation for this could be that rooms with factors allowing the endogenic growth of *Penicillium* will have a significant increase of spores in the air due to the abundant sporulation exhibited by this genus.

Finally, when we asked the hotel staff about guest distribution guidelines, two scenarios were found: hotels 1 and 2 assigned rooms starting from the bottom of the building, and hotel 3 assigned rooms starting from the top of the building. When we analyzed the fungal concentration considering these two alternatives, a positive correlation was found for each case, where higher fungal concentrations were found in the lower floors for hotels 1 and 2, and in the upper floors for hotel 3 (Figure 1D,E), corresponding to a higher spore concentration on floors with increased human activity.

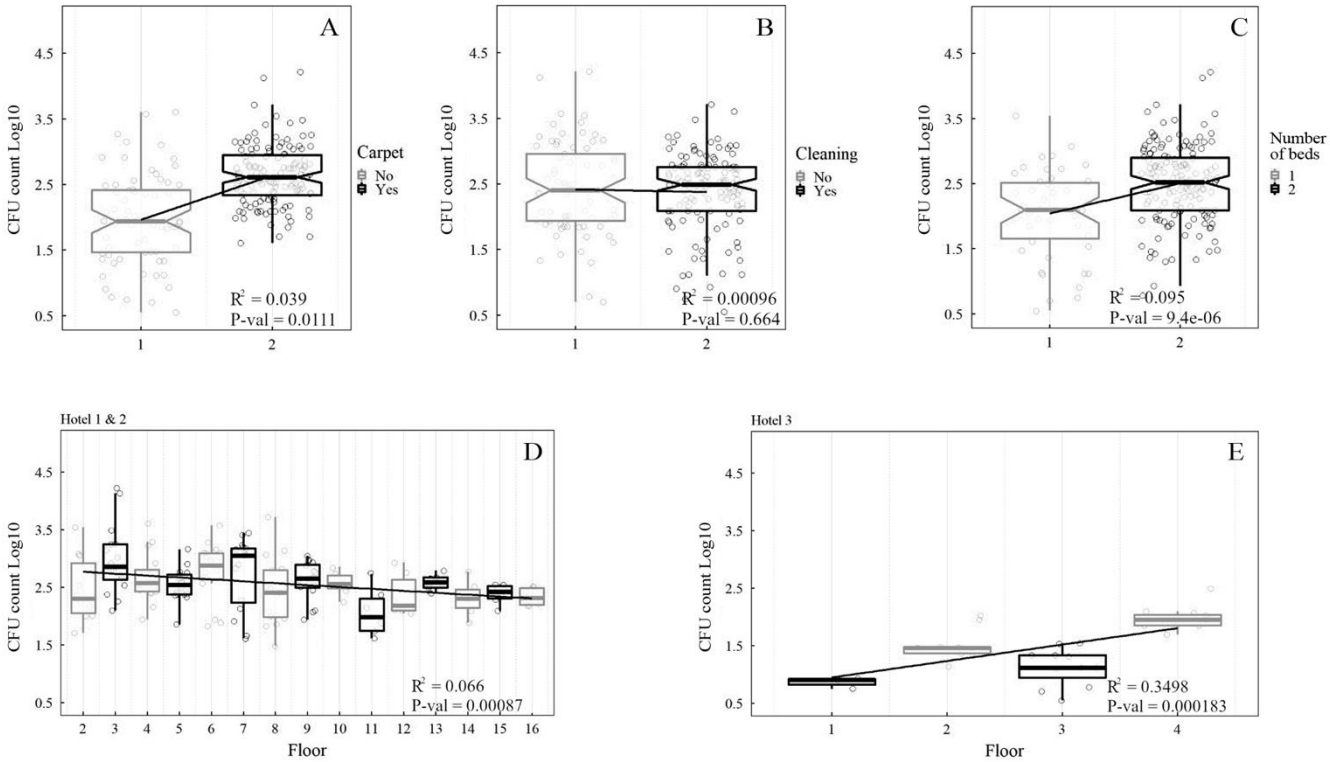

Figure 1. Statistical comparison of fungal CFU per M^3 among selected variables: rooms with carpet vs. rooms without carpet (**A**); rooms already cleaned vs. rooms not yet cleaned (**B**); rooms with one vs. two beds (**C**); floor number (**D**,**E**).

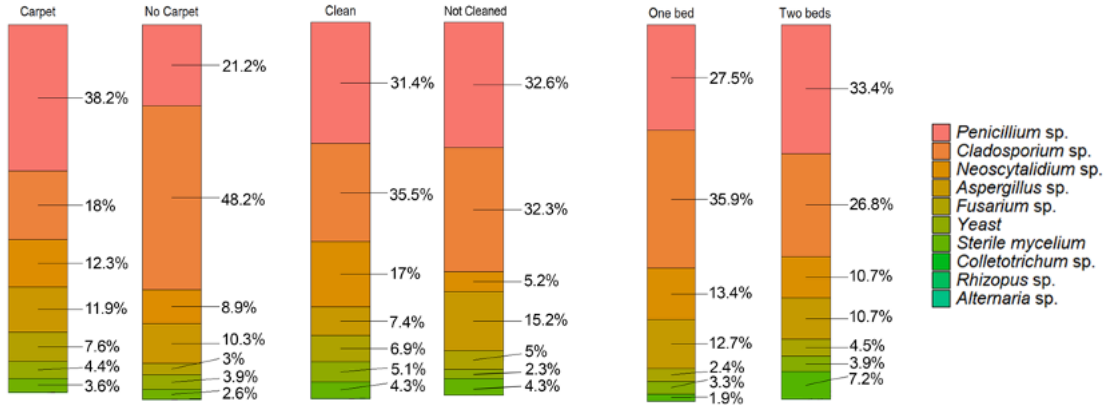

Figure 2. Fungi distribution (%) according to variable grouping (due to legibility reasons, the seven most abundant genera are shown).

4. Discussion and Conclusions

Fungal propagules are present in outdoor air throughout the year and thus, it is the main reason why it is impossible not to have spores inside houses and buildings. To our knowledge, no formal reports have been published in Mexico regarding fungal concentration in the air of hotel rooms. However, most Mexican studies on airborne fungi show Penicillium, Cladosporium and Aspergillus as the dominant genera in the air inside various types of buildings, with which our results agree [15–32].

Once spores from the outside are introduced to an indoor environment, regardless of vehicle, there are many factors that will influence their survival and multiplication, including temperature, moisture and substrate availability. Aside from these, there are many building-related factors that may influence the fungal presence in indoor air and it is

important to understand them in order to reduce human exposure and the related health risks [33,34].

For scenarios where water related damages or visible mold are not an issue, the presence of carpets in floors and/or walls is a factor well known for its positive correlation with higher fungal concentration [35,36], thus it is not surprising to find a significantly higher concentration of fungi in rooms where carpet is present in this study.

Human activity inside a closed space is a factor of variation in fungal concentration, even with a difference of hours [37]. Because of this, a difference between the concentration in cleaned and not cleaned rooms was expected, considering the activity generated by the housekeeping procedures. The difference found was not statistically significant and showed that despite recent human activity in them, cleaned rooms showed an overall trend towards a lower fungal concentration. On the other hand, a clear correlation can be made for the trends found between the method for room assignment and fungal concentration. This could be simply caused by the increased activity in those rooms, comprising more people coming and going with the consequent increase in dust [38].

The most surprising finding was the difference found between rooms with one and two beds; previous reports have not addressed something as specific and have even concluded that fungi in air cannot be reliably predicted by home characteristics [39,40]. It has been shown that mattresses along with carpets in non-problem dwellings and without moisture damage can provide a habitat with enough moisture to support fungal growth despite the lack of an obvious moisture source [41]. Based on this, having two beds vs. one bed allows for an increased surface for particle deposition/multiplication, which would explain a higher fungal concentration in air, especially if the room has the same dimensions. Two beds would suppose a higher human activity, which would result in more particles resuspended. If our results are supported by further studies, the number of beds become highly relevant not only for hotels but for homes and hospitals as well, in the prediction of fungal presence in air. These findings could be useful in updating protocols for cleaning or sanitization, or designing new protocols aiming for more efficient strategies that consider room characteristics. The detailed data we collected can be found in supplements material.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11156773/s1. The detailed data we collected can be found in supplementary.xlsx.

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