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Common fungi and major factors of the contamination of student dormitories indoor

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Abstract

***Background & Aims*:** Microbiological quality of environments is an important criterion that must be taken into account when indoor workplaces are designed to provide a safe environment. Therefore the purpose of this study is to provide insight into how students are exposed to fungal contamination of the dormitory indoor and to figure out the major possible factors that govern the contamination levels.

***Material & Methods*:** The study samples were obtained from two female dormitories of UMS University. The specimens were collected by using sterile swabs from indoor environments such as rooms, kitchens, washrooms /bathrooms, corridors and study rooms. A morphologic identification was performed using colony features and microscopic characteristics for the fungal isolates, and the findings were confirmed by PCR-RFLP molecular method.

***Results*:** Molds and yeasts were recovered from the indoor places including rooms, study room, kitchens and bathrooms from student life areas of the dormitories. A total of 160 swab samples yielded fungal growth. The number of fungal colonies recorded was 458 cps (colony per swab) included common mold: *A. flavus* (31.7%), *A. fumigatus* (28.7%), *A. niger* (5.8%) and yeasts: *Candida albicans, C. dubliniensis, C. krusei*. The black fungi (dematiaceus fungi) were totally isolated 67 (11.5%) followed by other molds included Penicillium (9.5%), Rhizopus (4.3%), Scopolariopsis (0.5%), Pseudoallescheria and Fusarium (0.35% each).

***Conclusion*:** Our findings show that Aspergillus species are most common fungi contaminant dormitories indoor and kitchens contain most species and numbers of molds.

***Keywords*:** Fungi, Contamination, Dormitory, Rush Hour, Free Time

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Introduction

Bio-aerosols in the indoor environment are the presumed or confirmed causative agents of various infectious diseases and their components are associated with the development and exacerbation of chronic respiratory illness including asthma and respiratory tract

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infections. Several studies have been indicated that the housing conditions, the human activities and life style of occupants are thought to be the principal factor contributing to the buildup and spread of bio-aerosols contamination the indoor environment. Other important sources of biological particulate matter may be human oral and respiratory fluid emitted via talking, sneezing, coughing, breathing and the direct shedding of skin related microbiota (1). A review made by WHO on the number of epidemiological studies showed that, there is sufficient evidence for an association between indoor dampness-related factors and a wide range of effects on respiratory health, including asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze and dyspnea (2). The presence of bacteria and fungi the indoor air poses a serious problem from the point of view of health protection and environmental engineering. Precise determination of various groups of microorganisms indoors is necessary; both to estimate the health hazard and to create standards for indoor air quality control. Four dormitories were sampled by "the air exposure method", using nutrient agar and sabouraud dextrose agar (3). There are excessive contaminant spores in indoor surfaces of dormitory rooms and human occupancy produces a marked spore concentration increase of most fungi species in UMS University dormitories. Thus the microbiological quality of environments is an important criterion that must be taken into account when indoor workplaces are designed to provide a safe environment. Therefore, the purpose of this study is to provide insight into how students are exposed to fungal contamination of the dormitory indoor and to figure out the major possible factors that govern the contamination levels.

Materials and Methods

Subjects:

The study samples were obtained from two female dormitories (Boostan and Gloestan) in UMS University, Urmia, Iran. Table 1 shows property terms of the dormitories, Boostan (A) and Golestan (B) as old and modern properties. A schedule was arranged for sampling in two sections, including busy time (rush hours) and free time of the dormitories. The specimens were collected by using sterile swabs from indoor environments such as rooms, kitchens, bathrooms, corridors and study rooms. The sample swabs were directly inoculated into fungal basic culture media sabouraud glucose agar (HIMEDIA MO63, India) and brain heart infusion agar (MERCK, KGaA64271, Darmastadt, Germany).

Identifications:

A morphologic identification was performed using colony features and microscopic characteristics for mold fungi and two differential culture media, CHROM agar Candida (HIMEDIA, M1297A, India) and corn meal agar (HIMEDIA, M146, India) were used for the isolated yeasts (4). As the most medically important fungi, *Candida* and *Aspergillus* species were confirmed by using molecular method; PCR-RFLP with restriction enzymes *Msp* I and *Mwo* I respectively (5-6). For the molecular identification, it was necessary to prepare Aspergillus mycelia mass purified for the DNA extractions. It was performed by making 12-24 hour's liquid cultures. Phenol –Chloroform method was used for the isolation of DNA. Each PCR reaction included a negative control which was 50 µl of double deionized water (DDW-MERCK) and also employed *C. albicans* (ATCC 10261, DNA extracted (boiling, phenol-chloroform method) as the positive control. The DNA fragments were subjected to electrophoresis system (including 1.5% agarose gel in Tris Borate EDTA buffer and post staining with ethidium bromide 0.50 mg/ml) (5). The PCR assay was performed using 5 µl of the DNA template in a total reaction volume of 50 µl consisting of PCR buffer (20 mM Tris- HCl at pH 8.0), 50 mM KCl, 0.1 mM each and 1.5U of *Taq* DNA polymerase. All PCRs were performed in a thermal cycler (Bioer XL, Fargene Poyesh, Iran). Thirty amplification cycles were performed after initial DNA denaturation at 95 °C for 5 min. Each cycle consisted of a denaturation step at 95 °C for 30 s, an annealing step at 55°C for 30 s, and an extension step at 72°C for 1 min, with a final extension at 72 °C for 5 min following the last cycle. PCR products were visualized under UV

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illumination to verify amplicon as the sharp band. Two restriction enzymes were used in RFLP produced differential patterns to identify some *Candida* and *Aspergillus* species (5, 7) isolated from the clinical and environmental specimens (Figure 1). The data obtained from morphological and molecular analyzed and compared between the busy time and free time samplings.

Results

Molds and yeasts were recovered from the indoor places including rooms, study room, kitchens and bathrooms from student life areas of the dormitories. A total of 160 swab samples yielded fungal growth. The number of fungal colonies recorded was 458 cps (colony per swab). All colonies were identified to molds and yeasts in species, genus and group levels. The species of Aspergillus as opportunistic pathogens found most frequent included *A. flavus* (31.7%), *A. fumigatus* (28.7%) and *A. niger* (5.8%). All of the Candida isolated (2.4%) were identified including *Candida albicans, C. dubliniensis, C. krusei*. The black fungi (dematiaceus fungi) were totally isolated 67 (11.5%) followed by other molds included Penicillium (9.5%), Rhizopus (4.3%), Scopolariopsis (0.5%), Pseudoallescheria and Fusarium (0.35% each) (Table 2). With the exception of bathrooms, all studied places were contaminated by Candida species and Scopolariopsis was only isolated from bedrooms. The kitchens included the most fungal contamination (174 colonies per swab). The lowest fungal isolations were recorded from the bathrooms (108 colonies per swab). *A.fumigatus* and *A. flavus* were the most common among all isolated fungi, *A.fumigatus* recovered from kitchens, bedrooms and bathrooms with 95cps, 39cps and 23 cps respectively. *Trichophyton mentagrophytis* isolated only 1 case from swab sample of a chair in a bedroom (Table 1). Among all places of sampling, kitchens and study rooms revealed the most fungal isolation (110 cps, 66 and 66 cps) from desks, garbage bins, oven stoves respectively (Table 3). Our results of comparing two dormitories revealed no significant difference between old and new structured dormitories for the contamination rate and frequencies of fungal isolation (p-value <0.05). The frequency of fungal isolations in two times of dormitory populations (busy time and free time) presented in Table 2. An unequal variance two-sample t-test was undertaken between the estimated coefficients for the different time-of-dormitory populations. As shown, the difference between the peak period of student presence and a free time period is statistically different to a 95% confidence level.

Table 1: Property terms of two studies dormitories (A: Boostan, B: Golestan)

Property Terms	Dormitory A	Dormitory B
Structure date	1993	1989
Operation date	1994	2008
Area	11000 m2	9000 m2
Foundation	6324 m2	5000 m2
Standard Capacity	360	240
Student number	364	250
Room numbers	98	64
Bathrooms showers	42	24
Basins numbers	43	32
Cooling system no.	98	64

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Table 2: Fungal contamination rate in the dormitories A and B, places and activity times based on fungal types

Fungi Contaminant	Dormitory A (Old)	Dormitory B (Modern)	Study rooms	living rooms	Kitchens	bathroom	No	Rush hours	Free times
Aspergillus fumigatus	7.52	6.60	11	39	95	23	168	36.38	15.13
Aspergillus niger	18.27	3.77	12	12	5	5	34	6.36	6.77
Aspergillus flavus	8.60	8.49	64	14	55	52	185	40.55	23.10
Trichophyton Mentagrophytis	0.17	0	0	1	0	0	1	0.17	0
Candida species	10.75	3.77	6	7	1	0	14	0.99	0
Penicillium sp	8.60	16.03	10	36	3	6	55	7.15	10.35
Rhizopus sp	9.67	3.77	8	8	6	3	25	1.19	13.14
Black fungi	17.20	48.11	18	31	6	12	67	3.57	20.71
Fusarium sp	1.07	0.94	0	1	0	1	2	0	0.79
Scopolariopsis sp	1.07	0	0	3	0	0	3	0.59	0
Pseudoallescheria sp	0	2.83	1	0	0	1	2	0.19	0
Other fungi	17.20	5.66	9	11	3	5	28	2.38	0
Total	100	100	139	163	174	108	584	100	100

Table 3: Frequency and percentage of fungal spores contaminating dormitories indoor places

Indoor samples	Fungal Contamination	
	No of CPS	%
study room desks	110	18.8
study room door knobs	17	3
study room walls	20	3.4
Study room floor	14	2.4
Bed room floor	33	5.6
Bed room blanket	28	4.6
Bed room sleepers	27	5.45
Bed room door knob	42	4.6
Bed room wardrobe	15	3.27
Bed room chairs	18	2.6
Kitchens all surfaces	66	11.3
kitchen water taps	29	6.33
Kitchen stove handles	13	4.9
Kitchen garbage	66	11.3
Bathroom walls	47	0.8
Bathroom floor	39	0.8
Bathroom door	8	6.6
Bathroom showers	14	2.4
total	584	100

CPS: Colony per swab

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Discussion

Different types of indoor environments (primary school, kindergarten, cafeteria, restaurant, dormitory, dwelling, office, sport salon, library, classroom, laboratory and their outdoor environments were investigated in terms of bio aerosol contamination (8).

Bio-aerosols in the indoor environment are the presumed or confirmed causative agents of various infectious diseases, and their components are associated with the development and exacerbation of chronic respiratory illness including asthma and respiratory tract infections. Several studies have been indicated that the housing conditions, the human activities and life style of occupants is thought to be the principal factor contributing to the buildup and spread of bio-aerosols contamination the indoor environment (1). The presence of bacteria and fungi an indoor air poses a serious problem from the point of view of health protection and environmental engineering. Precise determination of various groups of microorganisms indoors is necessary; both to estimate the health hazard and to create standards for indoor air quality control.

Four (4) dormitories were sampled by "the air exposure method", using nutrient agar and Sabouraud Dextrose Agar (2). Dormitories, the public places where some people with common jobs or activities live and rest together, could be a reason of some infectious diseases. Fungi including molds and yeasts both colonize at dormitories indoor and outdoor; Molds distribute around with very small spores and are able to infiltrate into the respiratory tracts of dormitory residents. Some other molds like pathogenic dermatophytes have the invasion to epiderm and skin surface. Although pathogenic yeasts such as *Candida* species colonize in mucosal and superficial layers.

In this study, different types of dormitory indoor environments (living rooms, study rooms, bathrooms and kitchens) were investigated for the fungal spores. A total of 36 environments were investigated in two dormitories. During the sampling, indoor temperature, relative humidity and CO2 were considered as the conserved conditions. Although in some studies, environmental factors were measured or redesigned because of the high number of environments (120 environments) investigated in terms of bio aerosol contamination (9). In the study of bacterial and fungal levels in various indoor and outdoor places by Mentese *et al*, the highest bacteria levels were measured in kindergartens, primary schools, restaurants, high schools, and homes, while the highest mold levels were measured in kitchens, bathrooms and offices. *Micrococcus* spp, *Staphylococcus auricularis*, and *Bacillus* spp. were predominant bacteria species and *Penicillium* spp., *Aspergillus* spp., and *Cladosporium* spp. were the most observed mold genera detected in the samples (9). Our findings of fungal levels of dormitories indoor are closely in agreement with above study so that *Aspergillus* species, *Penicillium* species and Black fungi (including Cladosporium spp.) had the highest level of isolations. A French prospective case-control study of 118 dwellings in Eastern France examined fungal contamination in unhealthy dwellings (n=32), dwellings occupied by allergic patients (n=27) and matched control dwellings (n=59). In that study, molecular identification of 12 *Penicillium* species showed *Penicillium chrysogenum* and *Penicillium olsonii* to be the two main species (8). In our study on two University students' dormitories, the most common molds were *Aspergillus flavus* and *Aspergillus fumigatus* followed by *Penicillium* species and black fungi. Aspergilli are worldwide molds have a potency to make allergic diseases and also life threatening infections in high- risk persons.

In Iran Aspergillus molds sourced by constructions and renovations and the spores distribute to various dwellings including dormitories. Our results also show that kitchens as one of the indoor places of dormitories had the highest isolation of molds including *A. fumigatus* and *A. flavus* in comparison with study rooms. In fact, accumulation of garbage, preserved foods as well as contaminated sinks and floors make produce a huge number of Aspergillus spores. Other places were living rooms and bathrooms with the high frequency of mold isolation including *Aspergillus fumigatus* and *Penicillium* species. In the study of Sybel *et al*, kitchens and bathrooms were the most contaminant places they

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measured by using molecular methods (9). There are the inconsiderable difference between two studied dormitories (old and new) for isolation of molds and yeasts, against difference existed among the terms of sampling so that, the range of fungi isolations is higher at the time of busy and high activities in the dormitories comparing the time of free and low activities.

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