

Original article

The relation between fungal propagules in indoor air and home characteristics

Background: Questionnaires are commonly used in epidemiologic studies to obtain information about house characteristics in order to predict the household aeroallergen exposure levels. However, the reliability of the predictions made with the questionnaires has not been evaluated. To address this issue, we compared objectively measured fungal propagules including the most frequently isolated mold genera (i.e., *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, etc.) in a large sample of homes and compared these measured values to the questionnaire-determined household characteristics.

Methods: As part of a prospective cohort study on the relation between residential allergen exposure and development of asthma in neonates, fungal air samples were collected from infant bedrooms and main living areas in 1000 homes in the Northeast USA, from December 1996 to January 1999. A Burkard portable air sampler was used in combination with DG-18 and MEA agars. A questionnaire was used to obtain information on host and house characteristics that may have an impact on the presence of fungal propagules in the air. This included information on observation of moisture problems (e.g., water leakage or damage, and mold or mildew growth), ventilation and heating facilities, building age and type, number of occupants, annual household income, presence of pets and pests, cleaning regimens, etc.

Results: The number of CFU/m³ air collected on MEA was significantly higher than on DG-18 (means, respectively, 1033.5 and 846.0 CFU/m³) ($P < 0.0005$). However, there was no significant difference between the numbers of CFU/m³ air collected from the main living area and from the infant bedroom. There was only a very weak relationship between the house characteristics, as described by questionnaire, and the presence of fungal propagules in indoor air. Only the temperature, relative humidity, season, and cats inside homes had a statistically significant impact on the presence of fungal propagules in indoor air.

Conclusions: The presence of fungal propagules in indoor air cannot be reliably predicted by home characteristics. Actual measurements are required for fungal exposure assessment, and the use of only one medium to collect samples in one location in a home might be adequate to represent residential levels of fungi in indoor air.

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There has been considerable concern about possible adverse health effects related to exposure to airborne materials of biologic origin. Molds are ubiquitous indoors as well as outdoors. The role of fungi in producing respiratory allergy and asthma has long been established (1–4). A number of recent epidemiologic studies have indicated a relation between home dampness/molds and respiratory symptoms (5–8). In

most of these studies, information on home dampness, mold growth, and respiratory symptoms was obtained from the occupants of the homes by means of questionnaires or observation, without objective exposure measurements of fungi levels. Therefore, the validity of this association has been questioned.

Fungi, which survive in favorable conditions in damp houses, are assumed to be validly assessed by subjective indicators such as damp spots, water leakage or damage report, and observation of mold growth. However, few studies have evaluated how well those surrogate indicators actually predict exposure to fungi in indoor environments.

As part of a prospective cohort study on the relation

Abbreviations. CFU/m³: colony-forming units per cubic meter; 95% C.I.: 95% confidence interval; DG-18: dichloran-18% glycerol agar; IB: infant bedroom; MA: main living area; MEA: malt extract agar; OR: odds ratio; SD: standard deviation; SE: standard error.

between residential allergen exposure and development of asthma in neonates, the presence of fungal propagules in indoor air was objectively assessed. The aims of the study were to investigate the applicability of this assessment as a measure of potential exposure to fungi, and to investigate the relation between the presence of fungi in indoor air and house characteristics. Additional objectives were to explore the representative sampling location in a home and to assess the performance of malt extract agar (MEA) and dichloran-18% glycerol agar (DG-18) in sampling and culturing target mold genera, and determining the concentrations of total culturable airborne fungal propagules.

Material and methods

Sample collection and analysis

Indoor air samples were collected from infant bedrooms and main living areas in 1000 homes in the Northeast USA, from December 1996 to January 1999. A Burkard portable air sampler (Burkard Manufacturing Co., Rickmansworth, UK) in combination with DG-18 and MEA was used to collect fungal airborne propagules. The sampling period was 1 min with an airflow rate of 20 l/min. After exposure, the plates were incubated at 25°C for 7–10 days, after which the resulting colonies were counted. Fungi were identified to level genus and, if possible, to species level by colony structure or microscopic examination of the spore structure. Concentrations were reported as colony-forming units per cubic meter (CFU/m³) of air. Temperature and relative humidity were recorded by Mannix digital thermo-hygrometer (Model LAM880D, Cole-Parmer Instrument Co., Vernon Hills, IL, USA) at the time of sampling.

Questionnaire

A questionnaire was used to characterize those variables of home environment and occupants' behavior which might have an impact on the presence of fungal propagules in indoor air. The questionnaire was administered in the home to an adult occupant of each home (typically, the mother of its index child followed in the health component of the study) by a trained research assistant. The questionnaires consisted of 64 items, including design and age of house; ventilation, heating, and air conditioning; humidifier and dehumidifier; type of flooring; observations of moisture problems (damp patches and/or mold or mildew growth on the walls, floors, carpets, or ceilings); the number of occupants; years of occupation; presence of pets and pests; cleaning routines; household income; and education level.

Statistical analysis

Differences between media or locations in individual mold genera and species as well as total concentrations were determined by the Wilcoxon signed rank test. Most variables were dichotomized with regard to their possible impact on the presence of fungal propagules in indoor air. A variable was given the value 0 if it was considered not to favor the development of molds in indoor air, and the value 1 if it was considered to favor mold development. The year was divided into three seasons because of the mild winters of 1996–1999. January to April was defined as winter/spring; May to August was defined as summer; September to December was defined as autumn. The total culturable number of CFU/m³ was categorized into three levels, according to the recommendation of the Commission of the European Communities (CEC) report (9). Fungal concentrations of less than 500 CFU/m³ were considered low, those between 500 and

999 CFU/m³ were considered intermediate, and those equal to or higher than 1000 CFU/m³ were considered high. The statistical analyses were carried with SAS software Version 6.12.

Results

Fungal genera and species

Large varieties of fungi were found among the 1000 home samples (Table 1). For approximately 4% of the samples, the count could not be made because of the high density of colonies on the agar plates. In total, 116 different mold species belonging to 44 genera were found. The most frequently isolated fungi were yeasts, which were counted in 89.3% of the homes. *Cladosporium* spp. were the second most frequently isolated molds, being present in 84.5% of homes. *Penicillium*, *Aspergillus*, and *Alternaria* were present in 72.6%, 50.3%, and 28.4% of the homes, respectively. *Epicoccum*, *Botrytis*, *Wallemia*, *Fusarium*, *Mucor*, *Curvularia*, and *Ulocladium* were present in less than 20% of homes (15.3%, 11.2%, 11.0%, 8.0%, 6.2%, 5.8%, and 5.1%, respectively). Unknown genera of fungi were found in 56.6% of homes, and all other fungi were found in 64.7% of homes.

Comparison of culturable fungal propagules between media and locations

The CFU/m³ in indoor air varied widely from home to home. The distributions (geometric mean, standard deviation, standard error, and range values) determined for culturable airborne fungi sampling results on DG-18 and MEA measured in both infant bedroom and main living area are summarized in Table 1. Significantly higher total culturable concentrations were observed on MEA than DG-18 independently of location (means respectively, 1033.5 and 846.0 CFU/m³) ($P < 0.0005$). However, no significant difference for total culturable concentrations between infant bedroom and main living area was observed regardless of the media used for sampling. Table 2 shows the cumulative distributions for concentrations of total fungi in indoor air at 0, 500, and 1000 CFU/m³, as well as the concentrations of the fungal genera *Alternaria*,

Table 1. Distribution of airborne fungal propagule concentrations in CFU/m³ in study houses

Media	Location	N	Mean	SD ^a	SE ^b	Min ^c	10%	25%	50%	75%	90%	Max ^d
DG-18	IB ^e	970	866.9	1655.0	53.1	0	0	50	300	900	2650	14 900
	MA ^f	973	825.2	1521.5	48.8	0	0	50	300	900	2300	14 300
MEA	IB	945	1029.0	1748.0	56.9	0	0	100	400	1200	4750	13 150
	MA	942	1038.1	1918.1	62.5	0	0	100	450	1150	4500	14 800

^a SD: standard deviation.

^b SE: standard error.

^c Min: minimal concentration in CFU/m³.

^d Max: maximal concentration in CFU/m³.

^e IB: infant bedroom.

^f MA: main living area.

Table 2. Cumulative distributions of culturable/viable airborne fungal propagules in CFU/m³

Media	Location CFU/m ³	Total fungi (%)			<i>Alternaria</i> (%)			<i>Aspergillus</i> (%)		
		0	≤500	≤1000	0	≤500	≤1000	0	≤500	≤1000
DG-18	IB ^a	10.5	61.5	76.4	88.4	100	100	71.6	97.3	98.5
	MA ^b	10.2	59.8	76.7	87.1	99.8	100	73.5	97.2	98.6
MEA	IB	8.1	53.5	70.6	90.4	100	100	82.5	99.2	99.7
	MA	7.9	52.4	70.9	91.3	100	100	84.1	99.0	99.6
		<i>Cladosporium</i> (%)			<i>Penicillium</i> (%)			Yeasts (%)		
		0	≤500	≤1000	0	≤500	≤1000	0	≤500	≤1000
DG-18	IB	36.6	86.7	94.4	57.5	91.6	93.7	57.3	93.0	94.9
	MA	37.3	84.5	93.6	58.1	93.0	94.8	54.1	94.2	95.8
MEA	IB	41.2	88.6	95.7	58.8	88.9	91.5	35.5	85.5	89.1
	MA	37.7	86.2	94.6	60.7	90.8	92.9	35.9	86.2	90.9

^a IB: infant bedroom.^b MA: main living area.

Aspergillus, *Cladosporium*, and *Penicillium*, and yeasts. No significant difference was observed in the cumulative distributions of total fungi between infant bedroom and main living area, or in the cumulative distributions of specific genera and yeasts ($P > 0.05$). For the different media used at the same location, significant differences in the cumulative distributions of total fungi, *Cladosporium*, and yeasts were observed ($P < 0.05$), but no significant difference in the cumulative distributions of *Alternaria*, *Aspergillus*, and *Penicillium* ($P > 0.05$).

The distributions (geometric mean, standard deviation, and range values) for the target mold genera and

yeasts on DG-18 and MEA at both infant bedroom and main living area are presented in Table 3. Significantly higher numbers of CFU/m³ were found on DG-18 than MEA for *Aspergillus* and *Cladosporium* ($P < 0.0001$) in both infant bedroom and main living area. For *Alternaria*, significantly higher numbers of CFU/m³ were measured on DG-18 than MEA agar in the main living area ($P < 0.05$), but not in the infant bedroom. Significantly lower numbers of CFU/m³ were found on DG-18 than MEA agar for yeasts ($P < 0.0001$) in both locations. No significant difference ($P > 0.05$) between DG-18 and MEA agar for *Penicillium* at either location

Table 3. Distribution of four targeted mold genera and yeasts in CFU/m³ in study houses

Genus	Media	Location	N	Mean	SD ^a	SE ^b	Min ^c	10%	25%	50%	75%	90%	95%	99%	Max ^d
<i>Alternaria</i>	DG-18	IB ^e	1000	9.1	35.5	1.1	0	0	0	0	0	0	0	100	700
		MA ^f	999	11.7	54.1	1.7	0	0	0	0	0	0	0	150	1150
	MEA	IB	1000	7.5	28.5	0.9	0	0	0	0	0	0	0	100	350
		MA	999	7.3	29.9	0.9	0	0	0	0	0	0	0	100	500
<i>Aspergillus</i>	DG-18	IB	999	74.2	365.3	11.6	0	0	0	0	0	100	250	1150	6950
		MA	999	77.7	375.8	11.9	0	0	0	0	0	100	250	1250	6100
	MEA	IB	1000	30.9	189.6	6.0	0	0	0	0	0	0	50	450	4500
		MA	998	31.8	232.3	7.4	0	0	0	0	0	0	50	500	4650
<i>Cladosporium</i>	DG-18	IB	999	238.7	463.8	14.7	0	0	0	0	200	600	1050	2050	5550
		MA	999	276.7	544.1	17.2	0	0	0	50	250	700	1200	3000	5300
	MEA	IB	997	230.6	635.5	20.1	0	0	0	0	200	550	900	3400	11 800
		MA	998	259.6	565.9	17.9	0	0	0	0	250	650	1050	3150	6550
<i>Penicillium</i>	DG-18	IB	985	274.2	1195.8	38.1	0	0	0	0	50	300	2350	14 650	14 650
		MA	987	227.4	1088.6	34.6	0	0	0	0	50	250	1150	13 600	13 600
	MEA	IB	981	326.7	1256.4	40.1	0	0	0	0	50	600	3450	15 300	15 300
		MA	981	342.1	1536.8	49.1	0	0	0	0	50	400	2550	14 800	14 800
Yeasts	DG-18	IB	985	208.7	929.3	29.6	0	0	0	0	50	250	1000	9900	9900
		MA	985	163.2	795.9	25.4	0	0	0	0	50	200	600	11 500	11 500
	MEA	IB	965	351.2	1032.2	33.2	0	0	0	0	200	1150	4450	8900	8900
		MA	962	320.6	1086.4	35.0	0	0	0	0	200	850	3900	13 700	13 700

^a SD: standard deviation.^b SE: standard error.^c Min: minimal concentration in CFU/m³.^d Max: maximal concentration in CFU/m³.^e IB: infant bedroom.^f MA: main living area.

Table 4. Relation between the total culturable number of CFU/m³ of indoor air from both infant bedroom and main living area and house-characteristics (logistic regression analyses)

Media	Location	Characteristics	High ^a vs low ^b			Median ^c vs low		
			OR ^d	95% C.I. ^e	P value	OR	95% C.I.	P value
DG-18	IB ^f	Summer vs winter/spring	2.480	1.467–4.194	0.0007	4.314	2.256–8.251	0.0001
		Autumn vs winter/spring	2.182	1.333–3.572	0.0019	3.413	1.850–6.296	0.0001
		Relative humidity (increase every 10%)	1.590	1.374–1.840	0.0001	1.523	1.290–1.799	0.0001
		Temperature (increase every 5°C)	2.252	1.656–3.061	0.0001	1.665	1.187–2.334	0.0031
		Presence of cat in home (yes/no)	1.796	1.172–2.750	0.0071			
		Presence of dog in home (yes/no)	1.532	1.037–2.264	0.0320			
		Use of wood burning stove (yes/no)				2.164	1.061–4.413	0.0338
	MA ^g	Presence of drapes and curtains (yes/no)				1.681	1.061–2.664	0.0270
		Summer vs winter/spring	3.518	2.041–6.066	0.0001	4.915	2.633–9.173	0.0001
		Autumn vs winter/spring	2.894	1.751–4.784	0.0001	3.999	2.233–7.163	0.0001
		Relative humidity (increase every 10%)	1.440	1.250–1.659	0.0001	1.473	1.263–1.718	0.0001
		Temperature (increase every 5°C)	2.061	1.514–2.805	0.0001	1.652	1.197–2.281	0.0023
		Presence of cat in home (yes/no)	2.395	1.538–3.727	0.0001	1.749	1.072–2.853	0.0252
		Presence of drapes and curtains (yes/no)	0.618	0.425–0.898	0.0116			
		Use of humidifier (yes/no)	0.626	0.442–0.888	0.0086	0.648	0.446–0.940	0.0223
		Molds and mildew formation (yes/no)	1.491	1.089–2.041	0.0126			
		Year of construction (after/before 1980)				0.572	0.351–0.931	0.0245
	IB	Summer vs winter/spring	2.182	1.369–3.478	0.0010	2.656	1.484–4.752	0.0010
		Autumn vs winter/spring	1.683	1.090–2.599	0.0188	2.470	1.458–4.185	0.0008
		Relative humidity (increase every 10%)	1.367	1.190–1.571	0.0001	1.502	1.283–1.760	0.0001
		Temperature (increase every 5°C)	1.992	1.494–2.658	0.0001	1.970	1.391–2.790	0.0001
		Presence of cat in home (yes/no)	1.928	1.293–2.876	0.0013	1.562	1.073–2.273	0.0199
		Use of air conditioning (yes/no)	0.607	0.435–0.845	0.0032	0.621	0.418–0.922	0.0180
	MA	Summer vs winter/spring	3.323	2.044–5.402	0.0001	2.153	1.254–3.696	0.0054
		Autumn vs winter/spring	2.022	1.304–3.135	0.0017	1.913	1.173–3.120	0.0093
		Relative humidity (increase every 10%)	1.452	1.272–1.659	0.0001	1.399	1.207–1.623	0.0001
		Temperature (increase every 5°C)	1.457	1.095–1.940	0.0099	1.768	1.286–2.432	0.0005
		Presence of cat in home (yes/no)	1.789	1.185–2.701	0.0056			
		Use of air conditioning (yes/no)	0.706	0.503–0.991	0.0440			
		Presence of spiders in past 12 months (yes/no)	1.685	1.184–2.400	0.0038			

^a High: fungal concentration ≥ 1000 CFU/m³.^b Low: fungal concentration < 500 CFU/m³.^c Median: fungal concentration between 500 and 999 CFU/m³.^d OR: odds ratio.^e 95% C.I.: 95% confidence interval.^f IB: infant bedroom.^g MA: main living area.

was observed. No significant difference ($P > 0.05$) between the two locations for *Alternaria*, *Aspergillus*, *Penicillium*, and yeast concentrations by either medium were observed. However, significantly higher concentration of *Cladosporium*, was found in the main living area than in the infant bedroom, independently of DG-18 or MEA collection medium used ($P < 0.0001$).

House characteristics and fungi

To investigate the relation between house characteristics and the total concentration of culturable/viable indoor fungi, we performed logistic regression analyses with 48 explanatory variables. Table 4 lists only those variables that had a significant ($P < 0.05$) influence on the CFU/m³ in indoor air by location and different media used for sampling. For the dependent variable of total concentration of fungi, the high vs low and the median vs low were modeled. For each variable, an

explanation is given for the situation in which it was considered to favor, or not favor, fungal propagules in indoor air. Furthermore, the odds ratios and 95% confidence intervals are presented. Independently of collection media (DG-18 or MEA) and location in the home (infant bedroom or main living area), season, relative humidity, and temperature were consistently found to be significantly related to the number of CFU/m³ indoor air. The presence of a cat in the home was also consistently and positively related to concentrations of culturable fungi in indoor air. Humidifier, air conditioning, wood-burning stove, presence of dog, spider, drapes or curtains, observation of mold and mildew, and year of construction were not consistently related to the concentration of fungal propagules in indoor air. The Hosmer and Lemeshow (HL) goodness-of-fit statistics ($P > 0.05$) were good enough to indicate that the multivariable (season, relative humidity, temperature, and presence of cat in home) model fits

the data well. However, R-square statistics, which are used to describe how well one can predict the dependent variable from the values of the independent variables, were all around 0.15 when data from different media and locations were analyzed.

The four common genera *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* were each categorized as present or absent. Both *Alternaria* and *Cladosporium* were strongly associated with season, showing a pattern of significantly higher concentrations in autumn and summer and lower levels in winter/spring ($P < 0.05$). The genus *Aspergillus* was also strongly related to season, but was only significantly higher in autumn ($P < 0.005$). No strong association was seen between the colony number of *Penicillium* and season. *Cladosporium* in indoor air was also highly associated with both temperature and relative humidity ($P < 0.005$). *Alternaria* in indoor air was significantly associated with temperature only ($P < 0.05$), not relative humidity. In contrast, concentrations of *Penicillium* in indoor air were significantly associated with relative humidity ($P < 0.05$), but not temperature. *Aspergillus* in indoor air was not significantly associated with either temperature or relative humidity. Like to total culturable fungi, some house characteristics, such as the presence of pets, mold and mildew formation, year of construction, and use of humidifier, could be related to the concentration of each specific fungal genus. However, those variables were not reliably predictors of the fungal concentration of each genus, because they were not consistently related to the number of each genus. Both the HL goodness-of-fit statistics ($P > 0.05$) and R-square statistics (around 0.05) in the specific genus models were similar to those models for which the total fungi number was used as the dependent variable. Although the models fitted well, as judged by the goodness-of-fit statistics, they had very low predictive power.

Discussion

Health effects caused by exposure to fungi in non-industrial indoor environments, including allergic reactions and the role of fungal metabolites, have been the focus of increased attention recently. Several ongoing epidemiologic studies are investigating the nature of the relationship between exposure to fungi and health effects. As part of a prospective epidemiologic study on the relation between residential allergen exposure and the development of asthma in neonates, we assessed indoor residential aeroallergen concentrations in 1000 homes. The exposure assessment protocol employed in the study permitted an evaluation of sampling methods for fungal propagules, the spatial distribution of concentrations within homes, and the utility of using questionnaire data to predict indoor

concentration. The results provide new information that should be considered in designing new studies aimed at assessing residential fungi levels.

Although significantly higher numbers of total culturable fungi were measured for samples collected on the MEA medium than the DG-18 medium, some specific genera, such as *Aspergillus*, *Cladosporium*, and *Alternaria*, had a significantly higher number of CFU/m³ on the DG-18 than the MEA medium. However, no difference for *Penicillium* between these two media was observed. These results indicated that DG-18 had superior performance on the counts of *Aspergillus*, *Cladosporium*, and *Alternaria*. DG-18, however, collected more diverse fungi than MEA. This finding generally agrees with the report by Russell et al. (10), suggesting that the use of only DG-18 to collect indoor air samples of fungi might be adequate to represent residential levels of those fungi which might be more directly related to health concerns. The advantages of using one medium for sampling are that it can save time, and be economical and practical.

No significant differences were found for the total number of culturable fungi as well as the numbers of the genera *Alternaria*, *Aspergillus*, and *Penicillium*, and yeast between the infant bedroom and the main living area. Only *Cladosporium* had a significantly higher concentration in the main living area than in the infant bedroom ($P < 0.0001$). The threshold concentration for evoking allergic symptoms for *Cladosporium* is 3000 spores per cubic meter air, which is much higher than the thresholds for *Alternaria*, *Aspergillus*, and *Penicillium* (11). More *Cladosporium* and *Alternaria* are fungi that grow mainly outdoors; *Aspergillus* and *Penicillium* mainly grow indoors. However, *Alternaria* is the most important outdoor mold and *Aspergillus* is the most important indoor mold (12). Therefore, our result suggests that it may be necessary to sample only in one location in a home in order to assess the household concentration of fungi if the allergenic species of fungi are of more concern. This apparently holds for measurements of both total and specific genera.

In this investigation, we documented an association between questionnaire reports of regular housing conditions and the concentrations of total culturable/viable fungal propagules and some common genera in indoor air by larger sample size (up to 3998 indoor air samples) than ever. Little information on the associations between home characteristics and fungal concentrations in indoor air is available in the published literature. In our study, strong and significant associations were found between the number of total culturable fungi in indoor air and season of the year, temperature, and relative humidity, as well as the presence of a cat in the home. However, the power was low to predict the dependent variable (airborne fungal concentrations) from the values of the independent variables (season of the year, temperature, relative humidity, and the

presence of a cat in the home). Damp spots, water leakage or report of water damage, observation of mold or mildew growth, etc. from questionnaires have often been used as surrogate measures for the number of fungi in several published epidemiologic studies (5–8). However, these characteristics were not consistently and significantly related to the measured culturable fungal propagules in indoor air in our study. Other questionnaire-obtained measures of house characteristics, such as the year of construction, use of air conditioner and humidifier, and heating system, were also not found to be predictors of fungal propagules. These results suggested that the presence of fungal propagules in indoor air cannot be reliably predicted by home characteristics. A similar conclusion was drawn by Verhoeff et al. (13), who found that the presence of fungal propagules in house dust could not be reliably predicted by house characteristics, as surveyed by a checklist and questionnaire. These results suggest that an actual measurement of fungal concentration in a home is more reliable than questionnaires for assessing residential exposure in epidemiologic studies. This finding may apply only to the Northeast USA, as this relationship may be different for different parts of country or world.

In this large sample, we further confirm that fungal concentrations show substantial variations or trends among seasons, as is consistent with our previous study (14). Summer and autumn were more likely to have higher concentrations of fungal propagules in indoor air than spring/winter. In addition, the seasons are associated with indoor temperature and relative humidity. Summer and autumn tend to have higher temperature and be more humid than spring/winter, which favor fungal growth. However, the question of how many samples in a year or in which season samples should be taken to best represent household fungal concentration is not clear. In our ongoing epidemiologic study on the relation between residential allergen exposure and the development and severity of asthma in childhood, we will assess how household fungal concentrations vary by season within and between homes.

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