Environmental Health and Preventive Medicine (2005.05) 10巻3号:157～161.

Indoor Airborne Mold Spores in Newly Built Dwellings
(新築住宅における室内空気中カビ胞子)

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Indoor airborne mold spores in newly built dwellings

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Running title: Airborne mold in newly built dwellings

Key Words: mold, sick building syndrome, *Cladosporium*, *Ulocladium*

Type of contribution: Short communication

Number of tables: 4

Number of figures: 0

Abstract

Objective: To investigate the relationships between sick building syndrome and mold in newly-built dwellings.

Methods: Symptoms of 61 residents in 18 dwellings were surveyed by standardized questionnaires. Mold sampling was done by gravity sampling using an open Petri dish. Potato dextrose agar (PDA) and dichloran-18% glycerol agar (DG-18) were used as the culture medium.

Results: There were 6 dwellings in which at least one inhabitant complained of one or more symptoms and 12 dwellings in which none of the inhabitants complained of symptoms. There was a tendency for the dwellings with inhabitants reporting symptoms to have larger colony forming units (CFU) on PDA than those without inhabitants reporting symptoms (p=0.1), but there was no difference in DG-18 result. There was a tendency for the dwellings with inhabitants reporting symptoms to have larger CFU of *Cladosporium* on PDA than those without (p=0.08), but there was no difference in DG-18 result. Significantly more *Ulocladium* sp. was detected in the dwellings with inhabitants reporting symptoms than in those without（P=0.03）. *Cladosporium cladosporioides* was detected in all the dwellings with inhabitants reporting symptoms and 75% of the dwellings without. *Cladosporium macrocarpum* and *Cladosporium herbarum* were detected in 33% of the dwellings with inhabitants reporting e symptoms and none of the dwellings without (P=0.1).

Conclusion: *Cladosporium* was dominant in the Japanese newly-built dwellings studied, and *Cladosporium* and *Ulocladium* were probably associated with the residents' symptoms in these newly-built dwellings.

Introduction

The indoor environment in dwellings is considered to be an important factor of health in many countries (1, 2). Many studies have reported that home dampness/mold in dwellings is related to symptoms, but many of these reports focused on dwellings such as relatively old multifamily buildings (3), or various types of and variously aged dwellings in Europe (4-6), or dealt with only respiratory symptoms (7-10). It may be considered that home dampness/mold is mainly a problem in old houses. However, as the airtightness of buildings has been increasing recently, a health risk due to dampness has arisen. We previously reported that in newly built dwellings dampness (condensation on window panes and/or walls, and mold growth) was significantly related to the symptoms that occurred in such dwellings (11). In Japan, over a million personal dwellings are newly constructed each year, therefore understanding the effects of mold in newly built dwellings is important. However, there are few reports on the relationship between symptoms and mold in such dwellings.

In this study, mold sampling was done by gravity sampling using an open Petri dish. We investigated the total amount of colony-forming units (CFU), genera and species of mold, and the relationships between symptoms and mold factors. We focused on eye symptoms, nose symptoms, skin symptoms, throat and respiratory symptoms and general symptoms that developed mainly in dwellings.

Subjects and Methods

Subjects

In our previous survey (12), we distributed questionnaires to residents of 564 dwellings (mainly solitary houses newly built or remodeled within the last several years) built by 24 construction companies in Sapporo and its environs. The questionnaires included queries about the building structures and characteristics (including condensation on window panes and/or walls, and mold growth), the residents' habits at home, and subjective symptoms. A total of 429 dwellings were in Sapporo and 135 were in the environs of Sapporo. The residents of 191 dwellings allowed measurement of levels of volatile organic compounds (VOCs) and aldehydes in their dwellings. We randomly selected 33 of 67 dwellings (49.3%) in which at least one inhabitant complained of one or more symptoms, and we also randomly selected 63 of 124 dwellings (50.8%) in which none of the inhabitants complained of symptoms. We measured the levels of VOCs and aldehydes at these 96 dwellings from August to September, 2001 in our previous study. The next year, we selected 11 dwellings in which at least one inhabitant had complained of one or more symptoms in 2001 and 7 dwellings in which none of the inhabitants had complained of symptoms in 2001, and we investigated the indoor occurrence of mold spores at the 18 dwellings from August to September, 2002. We also gave a self-administered questionnaire to the 18 families on the day of the mold investigation, and collected the questionnaires seven days later. If residents could not read or write, we requested the householder or partner to answer the questionnaires for them. This study was conducted with all the subjects' informed consent and approved by the institutional ethical board for epidemiological studies at Hokkaido University Graduate School of Medicine.

Questionnaire survey

Symptoms surveyed were as follows: eye symptoms (irritation of eyes, dry eyes, photophobia, lacrimation, and bloodshot eyes); skin symptoms (itching, dry, flushed , and erupted skin); nose symptoms (stuffy and runny nose); throat and respiratory symptoms (hoarsness, dry throat, cough, shortness of breath, and wheezing); general symptoms (fatigue, feeling heavy headed, headache, nausea, dizziness, and having difficulty concentrating). These symptoms were major sick building symptoms described in previous sick building reports (3, 13, 14). For each symptom, there were four alternative answers: “never,” “rarely,” “sometimes,” “usually.” There were three additional questions for each symptom, asking whether the symptoms occurred mainly in their dwellings, in the workplace/school, or anywhere.

The symptoms that occurred sometimes or usually mainly in the dwellings were defined as positive. A subject who had at least one positive for an anatomic site was designated positive for it.

The self-administered questionnaire given to the householder or partner contained queries about condensation on window panes and/or walls, mold growth in the dwelling, the age of the building, ventilation system, numbers of subjects living in the dwellings, type of dwelling (detached or duplex), building materials (wood or not), and pets at home.

Sampling procedure

Mold sampling was done by gravity sampling using an open Petri dish. Nine-cm Petri dishes containing potato dextrose agar (PDA) or dichloran-18% glycerol agar (DG-18) as culture media were used. One PDA petri dish and one DG-18 petri dish were for exposed 20 minutes in the living room in the daytime. The sampling was done under usual living conditions. Immediately after exposure the dishes were brought to the laboratory of the food microbiology section, of the Department of Microbiology, Hokkaido Institute of Public Health. After 5 to 10 days of incubation at 25^{o}C, mold colonies were counted and species were identified by their macroscopic and microscopic characteristics (15-19). The results were expressed as colony-forming units (CFU/plate/20min)

Statistical analysis

The Mann-Whitney U test was applied for analysis of associations between the symptoms and the CFU that were not normally distributed. Fisher’s exact test was used for analysis of associations between the symptoms and the detection rate of the mold species.

All the analyses were conducted using SPSS software for Windows version 10.0 (SPSS Inc., Chicago, U.S.A.).

Results

Initially, we selected 11 dwellings in which at least one inhabitant had complained of one or more symptoms and 7 dwellings in which none of the inhabitants had complained of the symptoms in the previous study in 2001. However, in this study, 5 of the 11 dwellings in which symptoms were reported shifted to the dwellings without inhabitants reporting symptoms, so there were 6 dwellings with inhabitants reporting symptoms and 12 without.

The age of the dwellings ranged from 1 to 5 years. There was no significant difference in the number of residents, building material, type of dwelling (detached or duplex), pets, ventilation, and dampness between the dwellings with and without symptoms reported (Table 1). In total, 61 residents answered the questionnaire and 6 of them had symptoms.

Table 2 shows the total amounts of CFU on the PDA and DG-18. There was a tendency for the dwellings where symptoms were reported to have more CFU on PDA than those without ($p=0.1$), but there was no difference in DG-18 results.

Table 3 shows the CFU of five genera of mold on PDA and DG-18. There was a tendency for the dwellings with inhabitants reporting symptoms to have more CFU of *Cladosporium* on PDA than those without inhabitants reporting symptoms ($p=0.08$), but there was no difference in DG-18 results. There was no difference in CFU results for mold genera.

Table 4 shows the rates of detected mold species. *Ulocladium* sp.. were detected significantly more often in the dwellings with inhabitants reporting symptoms than in those without them （P=0.03）. *Cladosporium cladosporioides* was detected in all the dwellings with symptoms reported and 75% of the dwellings without inhabitants reporting symptoms. *Cladosporium macrocarpum* and *Cladosporium herbarum* were detected 33% of the dwellings with inhabitants reporting symptoms but in none of the dwellings without them (P=0.1).

We also measured the concentrations of formaldehyde, acetaldehyde, and VOCs in their dwellings (data not shown). There was no dwellings in which the formaldehyde, toluene, p-dichlorobenzene, nonanal ethylbenzene, styrene and xylene concentrations exceeded the Japanese guideline value provided by the Japanese Ministry of Health, Labor and Welfare. The acetaldehyde concentration exceeded the Japanese guideline value (30 ppb) one dwelling with inhabitants reporting symptoms and three dwellings without inhabitants reporting symptoms (maximum 65ppb).

Discussion and Conclusion

Several investigators reported that signs of dampness were related to symptoms in dwellings (3-10). We also reported that signs of dampness related to symptoms in Japanese newly built dwellings (11). However there has been no report of relationships among sick building symptoms, the number of mold colonies, mold genus, and mold species in newly built dwellings. Therefore, this is the first such report, though our study had a small sample size.

We compared the total numbers of CFU. Dwellings in which residents had symptoms had marginally larger CFU on PDA, but not on DG-18. One previous report described positive associations between the symptoms of sick building syndrome and total numbers of CFU (20), but several other reports denied such associations (21-23).

In contrast, several studies reported positive associations between total numbers of CFU and specific diseases such as bronchial asthma [9, 24, 25].The reason why we found little relationship was possibly because the sample size was small and non-specific diseases were investigated.

The number of CFU of *Cladosporium* in the dwellings with symptoms reported was marginally larger than that in dwellings without inhabitants reporting symptoms (PDA; p=0.08, DG-18; p=0.15). As for the mold species, *Cladosporium cladosporioides* was detected in all the dwellings where symptoms were reported and in 75% of the dwellings where they were not.

Cladosporium macrocarpum and *Cladosporium herbarum* were detected marginally more in dwellings with inhabitants reporting symptoms (33%) than in dwellings without (0 %, P=0.1). *Cladosporium* is one of the most common mold genera in indoor and outdoor environments (26, 27). It has been reported that *Cladosporium* is associated with asthma (28), and lower respiratory tract illnesses (29). However, there has been no report of a positive association between sick building syndrome and *Cladosporium*. Meanwhile, a positive association between *Penicillium* and sick building syndrome in United States schools was reported (30). In this study, there was no association between *Penicillium* and symptoms. The reason for the marginally positive association with *Cladosporium* and the negative association with *Penicillium* is probably because the dominant genus of mold in Japanese newly built dwellings is *Cladosporium*, which the residents are mainly exposed to.

Ulocladium sp. were detected significantly more often in the dwellings where symptoms were reported. There has been no report of positive associations between *Ulocladium* and sick building syndrome or specific allergic diseases that develop mainly in the indoor environment. However, it was reported that *Alternaria* was associated with asthma (31) and lower respiratory tract illnesses (26). *Alternaria* and *Ulocladium* are morphologically closely related genera (32), so *Ulocladium* may possibly cause sick building syndrome or specific allergic diseases which develop mainly in the indoor environment, like *Alternaria*.

Health effects possibly caused by fungal exposure are classified into infections, allergies, toxic effects, irritation, and general symptoms (33). Sick-building syndrome is often caused by several environmental factors (13). Thus, a combination effect of fungal exposure and other environmental factors may be related to the symptoms in our study.

The present study has several limitations. First, for practical reasons, we used the open Petri dish method. This type of sampling relies on gravitational deposition of mold spores. A drawback to the open Petri dish method is the strong influence of wind and air turbulence, which means that larger spores might be overrepresented. However, in a comparative study, results obtained with the open Petri dish method showed good correlations with those obtained using a "Slitsampler" and a "N6 – Andersen Sampler," which both collect air into media at 30 l/min (34). Also, mold sampling in outdoor air could not be done, but we restricted the symptoms to those occurring mainly in the houses, so we could analyze the relationship between the symptoms and indoor air fungal exposure. Second, the reports on symptoms were only based on questionnaires. However objective diagnosis of sick-building symptoms is difficult, so the sick-building symptoms of many studies are based on questionnaires (3, 13, 14).Third, we selected 11 dwellings in which at least one inhabitant had complained of one or more symptoms in 2001, but, actually, only 6 dwellings where there were such complaints were examined in this study. We had explained the importance of ventilation of rooms at the end of the previous study, so increased attention to the indoor air environment possibly reduced their symptoms after the previous study. Thus, many subjects possibly had more concern about the indoor environment than the general population. Fourth, some chemicals may influence symptoms in the dwellings. We measured the concentrations of formaldehyde, acetaldehyde, and VOCs, and these concentrations were low. Fifth, we did not evaluate airborne fungi outdoors. *Cladosporium* is common in outdoor environments (26, 27), so outdoor airborne fungi may affect indoor environments. Finally, the sample size was small, and there was inadequate statistical power. Also we could not analyze the relationships between particular symptoms, other indoor air environment factors, and indoor airborne fungi. However, as mentioned, there has been no report on the relationship between sick building symptoms, the number of mold colonies, mold genus, and mold species in newly built dwellings. Therefore, to investigate the mold factors in newly built dwellings is very important.

Since this study was small and cross-sectional, further studies are needed, with a large population, and follow-up studies to clarify the relationships between particular symptoms, other indoor air environmental factors (bacteria, mite allergen, etc), and indoor airborne fungi, and interventional studies to confirm whether reduction of microbial growth in dwellings can diminish the symptoms of residents.

In conclusion, the dominant mold was *Cladosporium* in Japanese newly built dwellings, and *Cladosporium* and *Ulocladium* are probably associated with the symptoms of residents in newly built dwellings.

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Acknowledgment

This work was supported in part by a Health Science Research Grant from the Japan Ministry of Health, Labor and Welfare.

Table 1 Number of dwellings, with respect to characteristics of dwelling

	Symptoms, n=6		No symptom, n=12	
	Number of dwellings		Number of dwellings	
	n	(%)	n	(%)
Age of the dwelling (year)				
<1	0	0	1	8
1-<2	1	17	4	33
2-<3	3	50	3	25
3-<4	1	17	4	33
4-<5	1	17	0	0
Size of household				
1-2	1	17	4	33
3-4	5	83	6	50
5-8	0	0	2	17
Type of house				
Detatched	6	100	10	83
Terraced	0	0	2	17
Wooden house	6	100	9	75
Pets at home	1	17	3	25
Ventilation				
Natural ventilation only	2	33	2	17
Mechanical exhaust air only	3	50	7	58
Mechanical supply/exhaust air	1	17	3	25
Dampness				
Condensation on window panes and/or walls	4	67	6	50
Mold growth	3	50	7	58

Table 2 Total colony-forming units (CFU) by open Petri dish method

	Symptoms, n=6			No symptom , n=12			
	CFU/plate/20min			CFU/plate/20min			
	Median	Min	Max	Median	Min	Max	p value
PDA	8	1	14	4	1	8	0.10
DG-18	5	1	20	5	1	15	0.68

Table 3 Colony-forming units (CFU) of five mold genera by open Petri dish method

		Symptoms, n=6				No symptom, n=12				P value
		CFU/plate/20min			Identification	CFU/plate/20min			Identification	
		Median	Min	Max	rate	Median	Min	Max	rate	for CFU
Cladosporium	PDA	5	1	9	100	1.5	0	4	58	0.08
	DG-18	3.5	0	16	93	1.5	0	6	67	0.15
Alternaria	PDA	0	0	3	33	0	0	1	33	0.89
	DG-18	0	0	1	17	0	0	1	8	0.82
Aspergillus	PDA	0	0	1	17	0	0	1	8	0.82
	DG-18	0	0	0	0	0	0	1	17	0.62
Penicillium	PDA	0.5	0	2	50	0	0	1	8	0.15
	DG-18	0	0	2	17	0	0	1	8	0.75
Eurotium	PDA	0	0	0	0	0	0	0	0	1
	DG-18	0	0	1	17	0	0	2	8	0.82

Table 4 Detection rate of mold species on Petri dish by open Petri dish method

	Symptoms, n=6	No symptom, n=12	
	Detection rate (%)	Detection rate (%)	P value
Cladosporium cladosporioides	100	75	0.51
Cladosporium macrocarpum	33	0	0.1
Cladosporium herbarum	33	0	0.1
Cladosporium sphaerospermum	17	8	1
Cladosporium sp..	0	17	0.53
Alternaria infectoria	33	8	0.25
Alternaria alternata	0	33	0.25
Aspergillus penicillioides	0	8	1
Aspergillus amsterodami	0	8	1
Aspergillus ochraceus	17	0	0.33
Aspergillus sp.	0	8	1
Penicillium rugulosum	17	8	1
Penicillium paxilli	17	0	0.33
Penicillium simplicissimum	17	8	1
Penicillium corylophilum	17	0	0.33
Ulocladium sp.	50	0	0.03
Eurotium repens	17	8	1
Eurotium sp.	0	8	1
Epicoccum sp.	17	0	0.33
Stemphylium sp.	33	9	0.52
Botritis cimerea	17	17	1
Acremonium sp.	17	17	1
Phoma sp.	17	0	0.33

Arthrinium sp.	17	17	1
Nigerospora spherica	17	0	0.33
Mucor sp.	17	0	0.33
Yeast	50	25	0.34
Unidentified fungi	67	42	0.62