Tropical Biomedicine 24(1): 37–46 (2007)

Prevalence of *Penicillium s*pecific Ig E level and allergy symptoms among office workers in a selected company in Bangi, Malaysia

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Received 6 October 2006; received in revised form 12 February 2007; accepted 15 February 2007

Abstract. Indoor fungal reservoirs, particularly airborne *Penicillium* species, were identified throughout the ventilation system of the building and dissemination of fungi from those reservoirs was found to be occurring all the time. The objectives of this study were to determine the association between air concentration of indoor mould (*Penicillium*) and allergy symptoms among office workers. The study design used in this research was a cross-sectional study. Risk factors were identified through the questionnaire survey. Office workers were selected based on the proximity of their workstations to the microbiological air sampler used for the mould sampling. Results from the current study suggests that individual susceptibility of exposed subjects might be influenced by several factors associated with mould exposure; for example, inhaled mycotoxins or volatile organic compounds, which may, in some complex way, affect the immune response. This study provides the much needed preliminary baseline data for developing guidelines with validated findings that will be of use for policy decisions in Malaysia regarding indoor air quality. Results from this study are recommended for use in planning and implementing control measures in order to reduce the exposure to indoor mould and promote healthy working environment among the workers.

INTRODUCTION

Good indoor air quality is desired for a healthy indoor environment. Biological agents namely protozoa, mites, virus, and spores exist as important components influencing indoor air quality. Indoor air contains a complex mixture of bioaerosols such as fungi, bacteria and allergens, as well as non-biological particles including products from various combustion processes (Hargreaves *et al*., 2003).

Fungi are neither animals nor plants, fungi are classified in their own kingdom. It consists of moulds, yeasts, mushrooms, and puffballs. There are more than 100,000 accepted fungal species but current estimation range up to 10 million species (American Industrial Hygienist Association, 2005). Although the terms mould and fungi have been interchangeably referred to, all moulds are fungi, but not all fungi are moulds (AIHA, 2005). Some common indoor moulds are *Cladosporium*, *Penicillium*, *Alternaria* and *Aspergillus* (Centers for Disease Control and Prevention, 2005).

Fungi can cause disease in humans by a variety of biological mechanisms namely IgE-mediated allergy, fungal infection, irritant reactions, and toxic effects (Portnoy *et al*., 2005). For some people who are sensitive to moulds, exposure to them can cause symptoms such as nasal stuffiness, eye irritation, wheezing or skin irritation. Some may have more severe

reactions which include fever and shortness of breath. Some people with chronic lung illnesses, such as obstructive lung disease, may develop mould infections in their lungs (CDC, 2005). Elevated indoor concentrations of moulds might play a role in increasing the risk of developing atopic symptoms and allergic sensitisation not only to moulds but also to other common, inhaled allergens (Jacob *et al*., 2002). Indoor fungal reservoirs, particularly airborne *Penicillium* species, were identified throughout ventilation system of buildings and dissemination of fungi from those reservoirs was found to be occurring all the time (NIOSH, 1993; Malkin *et al*., 1998).

The objectives of this study were 1) to determine the level of air concentration of indoor mould (*Penicillium*) in the administrative office of this company; 2) to identify the prevalence of the *Penicillium* specific IgE and allergy symptoms among staff; 3) to determine the association between air concentration of indoor mould (*Penicillium*) and prevalence of *Penicillium* specific IgE; 4) to determine the association between air concentration of indoor mould (*Penicillium*) and allergy symptoms among office workers and 5) to determine the factors contributing to the prevalence of allergy symptoms after controlling the identified confounders.

Specific focus on *Penicillium* in this study was based on facts that sources of indoor Penicillium are various and can be associated with carpet, wallpaper, organic substances, and is also known to grow within fiberglass duct insulation (Forensic Analytical, 2005). *Penicillium* also is the most prevalent mould in the indoor environment, with the highest concentration of *Penicillium* accounted for 90% of the total fungi (Pastuszka *et al.*, 2000). A similar study by Carrer *et al*. (2001) also found moulds to be prevalent in indoor office environments. Exposure to elevated levels of *Penicillium* spores carries a risk of developing chronic allergic rhinitis, asthma, fungal sinusitis, and hypersensitivity pneumonitis. Allergy and asthma can only occur in individuals who can be genetically sensitised to *Penicillium* on exposure.

Results from this study are recommended for use in planning and implementing control measures in order to reduce the exposure to indoor mould and promote healthy working environment among the workers.

MATERIALS AND METHODS

This study was conducted in an office building situated in Bangi, Selangor. The company was established in 1992 as a main office with branches around the country. This location was chosen because it used centralised air conditioning unit and the floor was mostly carpeted inside the office. It had more than one hundred employees working in the indoor environment. The study design used in this research was a cross-sectional study Risk factors were identified through the questionnaire survey.

The air concentration of indoor mould was categorized into two categories namely higher than 35.00 cfu/m^3 and lower than 35.00 cfu/m^3. (The value 35.00 cfu/m^3 was the median of the air concentration of indoor mould, obtained after the measurement of indoor mould *Penicillium*).

There were four allergy symptoms studied in this research; 1) having red, itchy watery eyes; 2) having sneezing, congested runny nose; 3) having itchy or sore throat; and 4) having cough. Work duration was categorized into two categories namely, more than 40 hours per week and less than 40 hours per week.

Sampling

Study populations in this study were office workers on the three floors of the office building. The study sample consisted of office workers of all departments who were registered staff of the company. The list of names of respondents was obtained from the human resource department of the company based on their respective departments and locations.

A purposive sampling method was used. Office workers were selected based on the proximity of their workstations to the microbiological air sampler used for the mould sampling. Workers closest to the microbiological air sampler were selected first and the selection continued, in concentric circles from the location of the air sampler, until the required number of sample size was achieved. The inclusive criteria in this study were working in the company for at least 6 months (Malkin *et al.*, 1998), registered staff of the company age 18 – 56 years old.

Instruments

Questionnaire

A questionnaire based on modified Indoor Air Quality and Work Environment Symptoms Survey, NIOSH Indoor Environmental Quality Survey (1991) was used to obtain background information of the respondents. The questionnaire consisted of four sections namely Personal Information, Workplace Information, Information about Current Health Status, and Description of Workplace Conditions.

Measurement of indoor mould (*Penicillium*)

Air concentration of mould was measured using the Duo SAS Super 360 microbiological air sampler in 21 points in the office building. It employed the principle whereby the air-borne microorganisms were collected on microbiological agar by impaction produced by aspiration. The sampling time was 2 minutes (Hargreaves *et al.*, 2003) with sampling volume of 200 litres as recommended in the Duo SAS Super 360 microbiological air sampler manual (International Pbi S.p.a., 2003). The sampling media was malt extract agar (MEA) for *Penicillium* (NIOSH Manual of Analytical Methods, 1998; Samson *et al.*, 1994). Samples were taken in the centre of each 21 points, at the height of 1.1 m (Law *et al.*, 2000). Between the measurements the sampler was swabbed with 70% ethanol. The Petri dishes were incubated for 48 h at 25±2°C (Law *et al.*, 2000).

The calculation of mould (*Penicillium*) is based on the formula, X=(Pr x 1000) / V, shown in the Duo SAS Super 360 microbiological air sampler manual (International Pbi S.p.a., 2003), in which;

V = Volume of sampled air

r = Colony Forming Units counted on 90mm Petri Dishes

Pr = Probable count obtained from correction table to adjust colony counts from a 401-Hole Impactor Using Standard 90mm Petri Dishes

X = Colony Forming Units per 1000 litres ($1m^3$)

Blood sample analysis

Two ml of venous blood were drawn from selected respondents and inserted into test tubes with K_3EDTA. Samples were then centrifuged for 10 minutes using the Kubota 2100 centrifuge machine at 3000 rpm (round per minute) to separate the components. Then, the serum was separated carefully using a micropipette and inserted into vials. The vials were kept at -80°C in a freezer prior to human allergen (*Penicillium*) specific IgE ELISA blood assay. The Specific IgE ELISA Kit against *Penicillium chrysogenum* is an enzyme-linked immunosorbent assay for qualitative detection of allergen (*Penicillium chrysogenum*) specific human Immunoglobulin E. The principle of the procedure is solid phase capture sandwich ELISA assay using a microwell format. The range of detection is 0.35 – 0.70 Phadebas RAST Unit/ml (PRU/ml) while the reproducibility is C.V. 6% - 10%. The interpretation of the results was as follow:

a. 0.000 – 2.500 = Negative
b. 0.251 – 0.350 = Positive (Class I)
c. 0.351 – 0.450 = Positive (Class II)
d. 0.451 – 0.550 = Positive (Class III)
e. >0.550 = Positive (Class IV)

Quality control

Pre test was performed on 10% of the sample size to ensure the understanding of

the questions. The Duo SAS Super 360 microbiological air sampler was calibrated by the manufacturer before air sampling. Standard operational and sampling procedures were followed through the sample collection period. On the other hand, the Human Allergen Specific IgE ELISA kit is kept at 2° – 8°C. Standard operational procedure was followed. These include duplicating of samples.

Statistical methods

Data obtained from this study were analysed using Statistical Packages for Social Sciences (SPSS). Analysis was done using this software at different levels. Univariate, bivariate and multivariate testing were used to analyse variables in the study. The level of significance in this study was set at $p < 0.05$.

Study ethics

Approval from the Medical Research Ethics Committee, UPM was obtained. Besides that, permission from the employer of the office building where the study was conducted was also obtained. The respondents were informed about the purpose of the study through brochures because this study involved blood sampling while written consents were obtained from the respondents prior to the study.

RESULTS

Socio-demographic Characteristics (Table 1)

The mean age for the study group (n= 88) was 30.57 ± 5.90 years old. The composition of male workers was 44.3% while female workers were 55.7%. About 99.7% of workers were Malay while both Chinese and Indian comprised 1.1% each. There were 73.9% of workers who were married. In terms of education level, most of the workers were degree holders, comprising 61.4%. Workers with the lowest education level, UPSR, recorded 1.1% whereas 23 (26.1%) had SPM qualifications. STPM or Matriculation holders comprised 10 workers or 11.4%. The mean of work duration was 48 ± 2.48 years while the mean of work duration in a week was 42.57 ± 9.02 hours (Table 2).

Health information

Through the questionnaire, it was found that 35.2 % of the respondents had family history of allergy and 31.8 % had allergy to dust, 1.1 % had hay fever and about 6.8% allergy to moulds (Table 3).

Concentration of indoor mould and prevalence of *Penicillium* specific IgE and allergy symptoms

The mean of the air concentration of indoor mould was 28.07 ± 15.69 cfu/m^3. The median was 35.00 cfu/m^3 . The range of air concentration of indoor mould was

Table 1. Socio-demographic Characteristics of Respondents

Variables	Study group N = 88	
	Mean ± SD	n (%)
Age (years)	30.57 ± 5.90	–
Sex		
Male	–	39 (44.3)
Female	–	49 (55.7)
Race		
Malay	–	86 (99.7)
Chinese	–	1 (1.1)
Indian	–	1 (1.1)
Marital status		
Single	–	23 (26.1)
Married	–	65 (73.9)
Education level		
UPSR	–	1 (1.1)
SPM	–	23 (26.1)
STPM / Matriculation	–	10 (11.4)
University / College	–	54 (61.4)

Table 2. Work Duration of Respondents (n= 88)

	Mean ± SD
Work duration (years)	4.48 ± 2.48
Work duration in a week (hours)	42.57 ± 9.02

Table 3. Health Information of Respondents

Variables	Study group (N = 88) n (%)	
	Yes	No
Sinus infection	21 (23.9)	67 (76.1)
Asthma	6 (6.8)	82 (93.2)
Eczema	4 (4.5)	84 (95.5)
Hay fever	1 (1.1)	87 (98.9)
Allergy to dust	28 (31.8)	60 (68.2)
Allergy to moulds	6 (6.8)	82 (93.2)
Allergy to cats	7 (8.0)	81 (92.0)
Food allergy	20 (22.7)	68 (77.3)
Insect sting allergy	7 (8.0)	81 (92.0)
Medicine allergy	5 (5.7)	83 (94.3)
Family history of allergy	31 (35.2)	57 (64.8)
Other medical problems	11 (12.5)	77 (87.5)

Table 4. The Air Concentration of Indoor Mould

Variable	Mean	Median	Standard Deviation	Range
Air concentration of Indoor Mould (cfu/m^3)	28.07	35.00	15.69	0 – 70.00

0 to 70.00 cfu/m^3. The prevalence of *Penicillium* specific IgE among all the respondents was 72.73% (Table 4). The prevalence of allergy symptoms (red, itchy watery eyes; sneezing, congested nose; itchy or sore throat and cough) among all the respondents was 26.13%; 46.59%; 25.00% and 31.81% respectively.

There were no significant association between the air concentration of indoor mould and prevalence of *Penicillium* specific IgE among office workers (OR = 1.426, 95% CI = 0.556 – 3.658) (Table 5); between the air concentration of indoor mould and prevalence of allergy symptoms among office workers [red, itchy watery eyes (OR = 1.261, 95% CI = 0.484 – 3.283) (Table 6); sneezing, congested runny nose (OR = 1.604, 95% CI = 0.689 – 3.735); itchy or sore throat (OR = 0.694, 95% CI = 0.264 – 1.830) and cough (OR = 0.709, 95% CI = 0.288 – 1.744)]; between the prevalence of *Penicillium* specific IgE and prevalence of allergy symptoms among office workers red, itchy watery eyes [(OR = 0.612, 95% CI = 0.219 – 1.710) (Table 7); sneezing, congested runny nose (OR = 1.315, 95% CI = 0.510 – 3.394); itchy or sore throat (OR = 1.957, 95% CI = 0.587 – 6.521) and cough (OR = 1.571, 95% CI = 0.545 – 4.527)].

Of all the four allergy symptoms, only cough had a significant association with work duration in a week (OR = 2.877, 95% CI = 1.141 – 7.258) (Table 8). This means that the office workers who worked more than 40 hours in a week were approxi-

Table 5. Association between the Air Concentration of Indoor Mould and Prevalence of *Penicillium* Specific IgE among Office Workers

Variable		Indoor Mould Category		Odds Ratio	95% CI
		High (%) n = 46	Low (%) n = 42		
Prevalence of *Penicillium* Specific IgE	Yes	35 (76.1)	29 (69.0)	1.426	0.556 – 3.658
	No	11 (23.9)	13 (31.0)		

N = 88

Table 6. Association between The Air Concentration of Indoor Mould and Prevalence of Allergy Symptoms among Office Workers

Variable		Indoor Mould Category		Odds Ratio	95% CI
		High (%) n = 46	Low (%) n = 42		
Red, itchy watery eyes	Yes	13 (28.3)	10 (23.8)	1.261	0.484 – 3.283
	No	33 (71.7)	32 (76.2)		
Sneezing, congested runny nose	Yes	24 (52.2)	17 (40.5)	1.604	0.689 – 3.735
	No	22 (47.8)	25 (59.5)		
Itchy or sore throat	Yes	10 (21.7)	12 (28.6)	0.694	0.264 – 1.830
	No	36 (78.3)	30 (71.4)		
Cough	Yes	13 (28.3)	15 (35.7)	0.709	0.288 – 1.744
	No	33 (71.3)	27 (64.3)		

N = 88

Table 7. Association between The Prevalence of *Penicillium* Specific IgE and Prevalence of Allergy Symptoms among Office Workers

Variable		IgE Category		Odds Ratio	95% CI
		Yes (%) n = 46	No (%) n = 42		
Red, itchy watery eyes	Yes	15 (23.4)	8 (33.3)	0.612	0.219 – 1.710
	No	49 (76.6)	16 (66.7)		
Sneezing, congested runny nose	Yes	31 (48.4)	10 (41.7)	1.315	0.510 – 3.394
	No	33 (51.6)	14 (58.3)		
Itchy or sore throat	Yes	18 (28.1)	4 (16.7)	1.957	0.587 – 6.521
	No	46 (71.9)	20 (83.3)		
Cough	Yes	22 (34.4)	6 (25.0)	1.571	0.545 – 4.527
	No	42 (65.6)	18 (75.0)		

N = 88

Table 8. Association between Allergy Symptoms and Work Duration in A Week

Variable		Work Duration In A Week		Odds Ratio	95% CI
		> 40 hours n (%)	< 40 hours n (%)		
Red, itchy watery eyes	Yes	9 (25.7)	14 (26.4)	0.964	0.364 – 2.552
	No	26 (74.3)	39 (73.6)		
Sneezing, congested runny nose	Yes	18 (51.4)	23 (43.4)	1.381	0.586 – 3.254
	No	17 (48.6)	30 (56.6)		
Itchy or sore throat	Yes	8 (22.9)	14 (26.4)	0.825	0.304 – 2.238
	No	27 (77.1)	39 (73.6)		
Cough	Yes	16 (45.7)	12 (22.6)	2.877	1.141 – 7.258
	No	19 (54.3)	41 (77.4)		

N = 88

Table 9. Logistic Regression for Association between Cough Allergy Symptom and Work Duration In A Week

Variable		Work Duration In A Week		OR (95% CI)	*OR (95% CI)
		> 40 hours n (%)	< 40 hours n (%)		
Cough	Yes	16 (45.7)	12 (22.6)	2.877 (1.141 – 7.258)	3.266 (1.144 – 9.327)
	No	19 (54.3)	41 (77.4)		

N = 88
* Adjusted OR for age, food allergy, insect sting allergy, medication allergy, family history of allergy, having pets at home, smoking, URTI and other medical problems.

mately 2.9 times more likely to have the risk of developing cough than the office workers who worked less than 40 hours in a week (Table 9), After adjusted for age, food allergy, insect sting allergy, medication allergy, family history of allergy, having pets at home, smoking, Upper Respiratory Tract Infection (URTI) and other medical problems it was found that the office workers who worked more than 40 hours in a week were approximately 3.3 times more likely to have the risk of developing cough than the office workers who worked less than 40 hours in a week. (*OR = 3.266, 95% CI = 1.144 – 9.327).

DISCUSSION

Mean of the air concentration of indoor mould *Penicillium* was 28.07 cfu/m^3, geometric mean of 27.90 cfu/m^3 while the median was 35.00 cfu/m^3 and the range of 0 – 70 cfu/m^3. This finding is comparable with Hyvärinen *et al.* (1993) who stated that the level of *Penicillium* indoor air in the reference building had a geometric mean of 16 cfu/m^3 and the range of 0 – 72 cfu/m^3. The study also stated that the level of *Penicillium* in indoor air in buildings with mould problem was 31 cfu/m^3 (geometric mean) and between the range of 0 – 7900 cfu/m^3. Basically, the range of

Penicillium in indoor air in this study was similar to the reference building in the study done by Hyvärinen *et al.* (1993).

In this study, statistical analysis showed that there was no significant association between the air concentrations of indoor mould with the prevalence of *Penicillium* Specific IgE among office workers. However, Savilahti *et al.* (2001) reported significant association among 119 school children. Results from the current study suggests that individual susceptibility of exposed subjects might be influenced by several factors associated with mould exposure; for example, inhaled mycotoxins or volatile organic compounds, which may, in some complex way, affect the immune response.

There was also no significant association between the air concentrations of indoor mould with the prevalence of allergy symptoms among office workers in this study. The establishment of the etiology, the link between the exposure and the symptoms has until now been very difficult in such studies. This is because the knowledge on possible immunogenic and toxigenic effects on human beings following inhalation of non-proteinaceous (low-molecular) components from moulds is modest (Samson *et al.*, 1994). Katz *et al.* (1999) found that there was no significant correlation between the abundance of moulds and symptoms. However, Bornehag *et al.* (2001) in their review of 61 peer-reviewed articles stated that "dampness" in buildings appears to increase the risk for health effects in the airways, such as cough, wheeze and asthma with an OR in the range of 1.4–2.2.

The statistical analysis in this study also showed that there was no significant association between the prevalence of *Penicillium* Specific IgE with the prevalence of allergy symptoms among office workers. This was similar to the findings of Katz *et al.* (1999), whose study was based on 395 community populations in Israel aged 4 – 70 years old. The findings of this study however was not in line with that of Wallace *et al.* (1993), whose study was among 3948 employees of the Environmental Protection Agency in Washington DC in the winter of 1989. Wallace *et al.*(1993) stated that mould allergies were significantly associated with eye, nose, and throat symptoms; muscle pain; headache; fatigue; and dry skin. However, in Wallace's study, the association was a relation between perceptions of health and perceptions of the workplace. This in turn would produce bias and result in an apparent but false relationship between symptoms and workplace characteristics.

The insignificant result from this study may also be due to factors such as, a wide variation in the antigenic potency; reagents against many moulds were not commercially available and knowledge of the specific life stage or component of the mould that creates the sensitisation is limited as encountered by Storey *et al.* (2004). There were essentially two methods of testing for specific antibodies: skin testing and serum testing. Although they were both tested for specific IgE, there were some differences. Skin tests depended on the amount of IgE that was tissue-fixed on the mast cell, whereas the radioallergosorbant (RAST) and enzyme-linked immunoassay (ELISA) blood tests depended on the circulating IgE. However, since IgE has a high affinity for tissue, the concentration in the skin is greater and lasts longer, a matter of years, as opposed to circulation, which has a half-life measured in months (Storey *et al.*2004). The results of this study supports earlier observations and experiences indicating that the health effects of mould are not caused by IgE-mediated allergy to microorganisms but probably by other mechanisms (Husman, 1996; Savilahti *et al.*, 2001). According to Seuri *et al.* (2000), exposure of the workers in a military hospital building to mould and yeast in the indoor air caused an outbreak of occupational diseases, including asthma, rhinitis and alveolitis. The diseases were not immunoglobulin E (IgE)-mediated but might have been borne by some other, as yet unexplained, mechanism. Allergy symptoms are not chiefly caused by

exposure to mould. In fact, only 15% of those who were sensitive exclusively to mould, were symptomatic (Katz *et al.*, 1999). This statement was further supported by Bush *et al.* (2006) who mentioned that approximately 10% of the population have IgE antibodies to common inhalant moulds and that about half of these individuals (5% of the population) are predicted to have, at some time, allergic symptoms as a consequence of exposure to fungal allergens. Therefore, this indicates that mould sensitivity alone is not as important as the other allergens in inducing allergic symptoms.

After analyzing all the four allergy symptoms, only cough had a significant association with work duration in a week. A further analysis has found that the main factor that influences the prevalence of cough was the work duration in a week. The result of this study indicated that longer work duration in a week which meant more than 40 hours per week, would cause higher prevalence of cough allergy symptom. This was because longer duration spent in a work place with air-conditioning and carpet will probably induce cough among office workers. In previous study, it was found that all but one of the employees reported some building-related symptoms, the most common being a cough (Seuri *et al.* 2000). Besides that, living or working in buildings that are "damp" appears to increase the risk for a number of health effects mainly respiratory symptoms (cough, wheeze and asthma), but also other health effects such as unspecific symptoms like tiredness and headaches. Bornehag *et al.* (2001) in their review of 61 peer-reviewed articles concluded that the OR seems to be the highest for cough in terms of association with "dampness" and respiratory symptoms.

Mould spores can exist within dust. House dust is a heterogeneous mixture of substances, including emanations from dust mites, cockroaches, pets, and human subjects. It also contains viable and nonviable spores, mycelial fragments, fungal proteins, and byproducts of fungal metabolism (Portnoy *et al.*, 2005). Dust or otherwise known as particulate matter, had been proven harmful to the lungs. This can be identified through the reduction of lung function and the presence of persistent symptoms such as cough, phlegm, chest tightness, wheezing and breathlessness (Chan, 2003). Therefore, it could be possible that cough among the office workers in this study might be due to exposure to dust that contained mould spores; in short, dust was considered as a proxy of exposure.

This study provides the much needed preliminary baseline data for developing guidelines with validated findings that will be of use for policy decisions in Malaysia regarding indoor air quality. However much research needs to be carried on the health effects of indoor mould in relation to allergy and its link to asthma and other respiratory diseases.

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