Indoor Air 2017; 27: 24–33
wileyonlinelibrary.com/journal/ina

INDOOR AIR
doi:10.1111/ina.12278

Bacteria in a water-damaged building: associations of actinomycetes and non-tuberculous mycobacteria with respiratory health in occupants

Abstract We examined microbial correlates of health outcomes in building occupants with a sarcoidosis cluster and excess asthma. We offered employees a questionnaire and pulmonary function testing and collected floor dust and liquid/sludge from drain tubing traps of heat pumps that were analyzed for various microbial agents. Forty-nine percent of participants reported any symptom reflecting possible granulomatous disease (shortness of breath on exertion, flu-like achiness, or fever and chills) weekly in the last 4 weeks. In multivariate regressions, thermophilic actinomycetes (median = 529 CFU/m^2) in dust were associated with FEV_1/FVC [coefficient = −2.8 per interquartile range change, $P = 0.02$], percent predicted $FEF_{25–75\%}$ (coefficient = −12.9, $P = 0.01$), and any granulomatous disease-like symptom [odds ratio (OR) = 3.1, 95% confidence interval (CI) = 1.45–6.73]. Mycobacteria (median = 658 CFU/m^2) were positively associated with asthma symptoms (OR = 1.5, 95% CI = 0.97–2.43). Composite score (median = 11.5) of total bacteria from heat pumps was negatively associated with asthma (0.8, 0.71–1.00) and positively associated with FEV_1/FVC (coefficient = 0.44, $P = 0.095$). Endotoxin (median score = 12.0) was negatively associated with two or more granulomatous disease-like symptoms (OR = 0.8, 95% CI = 0.67–0.98) and asthma (0.8, 0.67–0.96). Fungi or (1→3)-β-D-glucan in dust or heat pump traps was not associated with any health outcomes. Thermophilic actinomycetes and non-tuberculous mycobacteria may have played a role in the occupants' respiratory outcomes in this water-damaged building.

J.-H. Park[1], J. M. Cox-Ganser[1], S. K. White[1], A. S. Laney[1,2], S. M. Caulfield[3], W. A. Turner[3], A. D. Sumner[2,4], K. Kreiss[1]

[1]Respiratory Health Division, National Institute for Occupational Safety and Health, Morgantown, WV, USA, [2]Vermont Department of Health, Burlington, VT, USA, [3]Turner Building Science & Design, LLC, Harrison, ME, USA, [4]University of Vermont Health Network Occupational Medicine, Berlin, VT, USA

Key words: Bacteria; Actinomycetes; Actinobacteria; Non-tuberculous mycobacteria; Fungi; Office buildings.

Ju-Hyeong Park
Division of Respiratory Disease Studies
National Institute for Occupational Safety and Health
1095 Willowdale Road, Morgantown, WV 26505, USA
Tel.: +304-285-5967
Fax: 304-285-5820
e-mail: gzp8@cdc.gov

Received for review 20 August 2015. Accepted for publication 23 December 2015.

Practical Implications

Occupants in damp buildings are simultaneously exposed to bacteria and fungi. In occupants of a water-damaged building, we found that levels of actinomycetes and mycobacteria measured by colony-forming units in dust were positively associated with respiratory health and levels of endotoxin in dust and drain tubing traps of heat pumps were negatively associated with respiratory health but that levels of fungi were not. Our study findings suggest that bacteria, including actinomycetes and mycobacteria, should be considered important exposures in epidemiologic studies of water-damaged buildings.

Introduction

Various adverse health effects from exposure to dampness in indoor environments are well acknowledged, although etiologic agents and mechanisms of individual diseases are poorly understood. The most important factor for indoor mold growth is moisture, but many bacteria also grow in these conditions. This indicates that occupants in damp buildings are simultaneously exposed to multiple microbial agents from both bacteria and fungi. This mixed exposure is likely to result in interactive effects among various microbial agents and produce more complicated health outcomes (Nevalainen and Seuri, 2005).

For the past two decades, mold exposure has been the major focus in regard to health effects among occupants in damp buildings, whereas bacterial exposure has been generally understudied (World Health Organization, 2009). However, bacteria may be as important as mold in health effects (Huttunen et al., 2003).

Bacteria are ubiquitous, but some genera of Actinobacteria may be more prevalent than others in indoor environments (Rintala, 2011; Rintala et al., 2008; Taubel et al., 2009; Torvinen et al., 2010). Actinomycetes, a group of filamentous fungi-like bacterial genera producing spores, are a subgroup of Actinobacteria that are recognized as a potential indicator of wet conditions (Nevalainen et al., 1991; Samson et al., 1994). Actinomycetes have been known as an etiologic agent of hypersensitivity pneumonitis (HP), such as farmer's lung disease (Lacey and Crook, 1988). These Gram-positive bacteria have also been associated with other adverse health effects among occupants of water-damaged buildings (Rintala, 2011).

Previously, we reported that the prevalence of sarcoidosis in current employees of the study building was elevated (three cases/136 = 2206/100,000) compared to the US white population (<1–40 cases/100,000); that post-occupancy asthma incidence was higher than pre-occupancy (11.5/1000 vs. 3.3/1000 person-years); and that prevalence of shortness of breath (prevalence ratio = 9.6), wheeze (9.1), and chest tightness (5.1) were higher compared to US office workers (Laney et al., 2009). We investigated the building to characterize microbial contamination in floor dust and condensate drains of heat pumps of the heating, ventilation, and air-conditioning (HVAC) system, and examined associations of exposure with occupants' health data.

Methods and materials

Study building

An office building with 136 occupants, located in the northeastern USA, was constructed as a single-story structure in 1978 with a three-story annex completed in 1991. The annex had more than 80 heat pumps in its HVAC system although the original building did not have them. The building had a history of water incursions through roofs, walls, and pipes, and some occupants had complained of indoor air quality problems. Health questionnaire and pulmonary function test (PFT) results documented an excess of various respiratory illnesses (Laney et al., 2009).

Health study

The epidemiologic study was approved by the State Health Department Review Board. We conducted a self-administered health questionnaire survey for all occupants who had spent 20 h or more per week in the building. By completing the questionnaire, participants indicated their consent for the participation in the study. We defined any asthma symptom as at least one of the following weekly in the last 4 weeks: wheeze, attack of shortness of breath, chest tightness, attack of cough, or awakened by breathing difficulty. Epidemiologically defined asthma (Epi-asthma) was defined as three or more asthma symptoms weekly in the last 4 weeks or current self-reported physician-diagnosed asthma. We defined any symptom reflecting possible granulomatous disease (granulomatous disease-like symptom) as one or more of the following weekly in the last 4 weeks: shortness of breath when hurrying on level ground or walking up a slight hill, fever and chills, or flu-like achiness or achy joints.

We offered spirometry and methacholine challenge tests to all occupants after obtaining written informed consent. Bronchial hyper-responsiveness was defined as a PC_{20} of ≤4.0 mg/ml (bronchial hyper-responsiveness) or a PC_{20} of 4.1–16.0 mg/ml (borderline). PC_{20} is the provocative concentration of methacholine that causes an interpolated 20% decline in FEV_1 from the baseline. Details of PFT procedures and results are presented elsewhere (Laney et al., 2009).

Environmental study

The building was vacated in February 2007. Building engineers conducted a comprehensive building diagnosis including leak testing, tracer gas testing, and pressure mapping, and evaluation of the HVAC system (Turner Building Science & Design, unpublished data, 2007). In April, we collected condensate flexible drain tubes from all accessible heat pumps in the ventilation system and heat recovery units on the rooftop. We separated sludge and liquid inside the tube for analysis. In June 2007, we divided the building areas into four sections based on the number of occupants and selected 30 formerly occupied rooms from each section, for a total of 120 rooms (47%). For rooms that had no exterior walls, we vacuumed a 2 m^2 area of floor around each workstation for 5 min. For rooms with exterior walls, we vacuumed a 2 m^2 area of floor around each workstation for 2.5 min and 0.1 m^2 from the floor along the edge of exterior walls for 2.5 min. For each dust sample, we used a polyethylene filter sock (Midwest Filtration Company, Fairfield, OH, USA) with a pre-cleaned crevice tool on a L'il Hummer™ backpack vacuum sampler (100 ft^3/min, 1.5 horsepower; Pro-Team Inc., Boise, ID, USA). We homogenized collected dusts by rotating on a 360-degree rotary arm shaker at 65 r.p.m. for 2 h and partitioned the samples after hair, fluff, and other larger objects had been removed (Park et al., 2006).

We analyzed each sample for (1→3)-β-D-glucan, endotoxin, and culturable microbes: total fungi with speciation; total bacteria with identification of the top three genera; Gram-positive and negative bacteria; actinomycetes (room temperature, mesophilic, and thermophilic); and non-tuberculous mycobacteria. We used the *Limulus* amoebocyte lysate assay to analyze for (1→3)-β-D-glucan (Shogren and Park, 2011) and endotoxin (Milton et al., 1992). We sent dust, liquid,

and sludge aliquots for analyses of culturable fungi and bacteria to EMLab P&K (Cherry Hill, NJ, USA). Serially diluted sample aliquots were spread onto malt extract, dichloran glycerol 18, and cellulose agars at room temperature for fungi, and trypticase soy agar (TSA) with 5% sheep blood for total bacteria, colistin nalidixic acid (CNA) agar for the selection of Gram-positive bacteria, and MacConkey agar for the selection of Gram-negative bacteria and incubated for 7–10 days. Fungal species were identified by mycologists based on morphological and physiological characteristics. Bacterial species were identified using gas-chromatography analysis of cellular fatty acid methyl ester profile (Sherlock Microbial Identification System; MIDI, Inc., Newark, DE, USA). Samples were cultivated on TSA for room temperature (20–26°C), mesophilic (35–37°C), and thermophilic actinomycetes (50°C) for 10 days. Actinomycetes were identified via characteristic microscopic appearance. Dust aliquot for non-tuberculous mycobacteria was suspended in sterile dilution water with Tween-20 containing 50 μg/ml of nystatin that suppresses fungal growth. Four serial dilutions of the suspension were initially cultured on Middlebrook 7H9 broth with glycerol at 35°C for 8 weeks, and then, all suspected colonies were subcultured to Middlebrook 7H11 selective agar for 5–7 days. Non-tuberculous mycobacteria were identified based on Kinyoun acid-fast staining and various substrate tests, including nitrite and nitrate utilization tests, at different temperature between 28 and 37°C.

Statistical analysis

We assigned half the limit of detection (LOD) to non-detectable samples, except for thermophilic actinomycetes and mycobacteria in liquid and sludge collected from drain tubes of heat pump system. For these analytes, we did not examine associations with health outcomes due to too few number of detectable samples. For floor dust samples, we calculated microbial loads in unit area of floor (unit/m^2) by multiplying the resulting unit per gram value by the total amount of dust collected in each floor sample and then dividing by a total area sampled from floor (m^2). Distributions of the microbial loads were right skewed, and log-transformed results were used in epidemiological data analysis. We calculated the geometric mean (GM) and geometric standard deviation (GSD) of each microbial agent. Health outcome variables included granulomatous disease-like symptoms, Epi-asthma, asthma-like symptoms, spirometry results, bronchial hyper-responsiveness, and the number of work days missed due to respiratory and non-respiratory illnesses. We used multivariate linear or logistic regression models to examine associations between health and individual microbial agents in dust or drain tubes, and the models were adjusted for age, gender, race, smoking status, and building tenure. To examine nonlinearity of exposure–response relationships, we used generalized additive models with a B-spline function with four degrees of freedom. From the logistic regression models, we reported the odds ratio (OR) and 95% confidence interval (CI) per interquartile range (IQR) or one composite score increase in exposure. Correlations among microbial agents were examined using Pearson correlation coefficients. As measurements in sludge and liquid samples from the same drain tube representing one exposure were not normally distributed with some extreme values, we constructed a composite exposure variable for each tube sample. First, ascending-ordered concentrations for the sludge and liquid samples for each agent were divided into ten equal parts and coded from 1 to 10 to generate decile rankings. Then, we summed the decile ranks from sludge and liquid for each agent. The composite scores were treated as continuous variables in the multivariate regression models. We used information on areas served by the heat pump and heat recovery units to match condensate tube samples and dust samples within the annex. We assigned microbial measurements in floor dust collected from the selected room to its occupant as an exposure. We considered $P < 0.05$ as statistically significant and $P < 0.10$ as marginally significant. All analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Microbial contamination in floor dust

Except for *Brevundimonas* species (Gram-negative), all the top three bacterial genera identified from each dust sample were Gram-positive. These genera included *Arthrobacter, Bacillus*, *Brevibacterium, Cellulomonas, Cellulosimicrobium, Kocuria, Microbacterium, Micrococcus, Ralstonia, Rhodococcus,* and *Staphylococcus*. Of these, *Bacillus* was the most prevalent (98%), followed by *Micrococcus* (33%). The levels of Gram-positive bacteria were extremely variable (GSD = 27.6) (Table 1).

Actinomycetes were detected in 74% of dust samples, and thermophilic actinomycetes were most predominant (81%) among the three types. Non-tuberculous mycobacteria were cultured from 23 dust samples (19%) with a maximum level of 190,000 CFU/g. Rapidly growing mycobacteria (RGM) were identified from 20 samples and slowly growing mycobacteria (SGM) from the remaining three. Isolated individual species included *Mycobacterium aichiense* (RGM), *Mycobacterium fortuitum* (RGM), *Mycobacterium saskatchewanense* (SGM), and *Mycobacterium intracellulare* (SGM). All samples analyzed were detectable for endotoxin, whereas only 46% of the samples were above the LOD for culturable Gram-negative bacteria.

Table 1 Levels and load of bacteria and fungi in floor dust ($n = 120$)

Microbial agent analyzed[a] (Number of samples >LOD)	Concentration (per gram dust)			Load (per m^2 floor)		
	GM (GSD)	Minimum	Maximum	GM (GSD)	Minimum	Maximum
Bacteria						
Total bacteria ($n = 120$), CFU	2.1×10^5 (9.0)	2800	5.4×10^9	9.3×10^4 (9.9)	690	2.3×10^9
Gram positive ($n = 92$)	9.8×10^3 (27.6)	100[b]	1.4×10^9	4.3×10^3 (30.1)	27	7.8×10^8
Gram negative ($n = 55$)[c]	590 (6.3)	100[b]	6.0×10^6	260 (7.1)	18	2.5×10^6
Total actinomycetes ($n = 89$), CFU	850 (4.0)	110[b]	2.9×10^4	370 (4.4)	18	1.6×10^4
Room temperature ($n = 17$)[c]	230 (2.4)	100[b]	1.3×10^4	98 (2.8)	18	6.6×10^3
Mesophilic ($n = 31$)[c]	250 (2.3)	100[b]	8.7×10^3	110 (2.5)	18	2.9×10^3
Thermophilic ($n = 72$)	580 (3.8)	110[b]	2.9×10^4	250 (4.1)	18	1.6×10^4
Mycobacteria ($n = 23$)[c], CFU	640 (2.5)	360[b]	1.9×10^5	290 (3.0)	58	9.7×10^4
Endotoxin ($n = 117$), EU	6.1×10^4 (2.2)	5800	7.4×10^5	2.8×10^4 (2.5)	2600	4.3×10^5
Fungi						
Total fungi ($n = 120$), CFU	4.1×10^4 (7.7)	260	3.8×10^7	1.8×10^4 (8.1)	46	2.1×10^7
(1→3)-β-D-glucan ($n = 118$), ng	4.3×10^4 (2.2)	970	1.2×10^6	1.9×10^4 (2.4)	1400	5.5×10^5

[a]The number of samples analyzed is 120, except for (1→3)-β-D-Glucan ($n = 118$) and endotoxin ($n = 117$).

[b]Half of the LOD was assigned to non-detect samples.

[c]The GMs (GSDs) for only samples above the LOD are 2700 (6.0) CFU/g and 1200 (6.9) CFU/m^2 for Gram-negative bacteria, 1200 (3.8) and 670 (3.5) for room temperature actinomycetes, 720 (2.7) and 290 (2.8) for mesophilic actinomycetes, and 2500 (4.0) and 960 (4.2) for Non-tuberculous mycobacteria.

Table 2 Levels of bacteria and fungi in liquid and sludge collected from heat pump system condensate drains

Microbial agent analyzed	No. of samples, Detected/total	Concentration in liquid (per ml)			No. of samples, Detected/total	Concentration in sludge (per gram)		
		GM (GSD)	Minimum	Maximum		GM (GSD)	Minimum	Maximum
Bacteria								
Total bacteria, CFU	64/67	1.3×10^5 (41.4)	5	8.1×10^8	49/68	4.2×10^5 (279.4)	25	7.3×10^8
Thermophilic Actinomycetes[a], CFU	0/67	– (–)	–	–	2/68	68 (1.3)	38	240
Mycobacteria[a], CFU	3/65	1.2×10^3 (54.9)	20	6.0×10^4	1/68	3.0×10^5 (–)	3.0×10^5	3.0×10^5
Endotoxin, EU	67/67	23 (11.7)	0.2	3.0×10^3	67/67	1.6×10^4 (7.1)	121	4.3×10^5
Fungi								
Total fungi, CFU	66/67	5.2×10^3 (7.4)	50	3.9×10^5	67/68	1.2×10^6 (5.7)	100	1.4×10^7
(1→3)-β-D-glucan, ng	67/67	15 (5.1)	0.5	3.3×10^3	68/68	5.9×10^4 (3.1)	3.1×10^3	8.4×10^5

For non-detect samples, we assigned half the LOD to compute geometric mean and standard deviation.

[a]For these microbial agents, no values were assigned to non-detect samples because there were too many below the LOD. Only samples above the LOD were included in the calculation.

Concentration of culturable fungi was more variable than that of (1→3)-β-D-glucan. The most frequently identified fungal species in dust included *Penicillium chrysogenum*, *Cladosporium sphaerospermum*, *Phoma coelomycetes*, and *Epicoccum nigrum* (Table S1). Correlations among microbial measurements in dust were mostly weak or moderate (correlation coefficients: 0.06–0.55).

Microbial contamination in condensation drain tubes

The heat pumps served all areas in the three-story annex for heating, ventilation, and air-conditioning. We analyzed liquid or sludge samples from 68 drain tubing traps from heat pumps. Of these, 66 samples were positive for total culturable bacteria in either the liquid or sludge (Table 2). Concentrations were extremely variable, ranging from non-detectable to 730 million CFU/g of sludge or 810 million CFU/ml of liquid. The top three bacterial genera identified for each sample were mostly Gram-negative and these included the following: *Acidovorax*, *Brevundimonas*, *Burkholderia*, *Chryseobacterium*, *Delftia*, *Methylobacterium*, *Pseudomonas* (*aeruginosa* and *fluorescens*), *Rhodopseudomonas*, *Sphingomonas*, and *Wautersia*. Of these, *Sphingomonas* was most prevalent (77.9%), followed by *Methylobacterium* (36.8%). Gram-positive bacteria were also among the top three genera identified for each sample, and these included *Bacillus* and *Brevibacterium*.

We found low concentrations of thermophilic actinomycetes in two sludge samples. Three heat pumps were positive for non-tuberculous mycobacterial cultures, and *Mycobacterium chelonae* and *Mycobacterium avium* complex (MAC) were isolated. The drain tubing traps from seven heat recovery units had four positive results for non-tuberculous mycobacteria [arithmetic mean = 420 CFU/ml of liquid (s.d.,

standard deviation = 28); 2.6×10^6 CFU/g of sludge (s.d. = 2.4×10^6)] (data now shown). The GM of culturable fungi in sludge was slightly higher than that in floor dust, while the level of (1→3)-β-D-glucan was similar.

The most frequently identified fungal species in dust were rarely found in drain tubes (Table S1). *Paecilomyces lilacinus*, *Penicillium corylophilum*, and *Penicillium pururogenum* were most frequently found in drain tubes, but were isolated in floor dust from only 2, 8, and 5 rooms, respectively. *Aspergillus versicolor* was most commonly found in both floor dust and drain tubes. However, there were only four rooms where the same species were identified from both floor dust and drain tubes of heat pumps that served the rooms. These species included *Aspergillus versicolor*, *Paecilomyces lilacinus*, *Penicillium corylophilum*, and *Penicillium pururogenum*.

Respiratory health outcomes associated with microbial contaminants in floor dust

Of the 136 building occupants, 77% (n = 105) participated in the questionnaire survey, 64% (n = 87) in spirometry and methacholine challenge tests, and 49% (n = 67) in both the questionnaire and spirometry and methacholine challenge tests. The majority of questionnaire participants were female (63%) and white (97%), and approximately half were either former or current smokers (48%). The mean age and tenure were 46.4 and 8 years, respectively (Laney et al., 2009). Half of the questionnaire participants reported having at least one granulomatous disease-like symptom weekly in the last 4 weeks. Of the three granulomatous disease-like symptoms, shortness of breath on exertion (33%) and flu-like achiness (32%) were more prevalent than fever and chills (6%). Prevalence of Epi-asthma was 24%, and there were 13 participants with abnormal spirometry (nine with obstruction, two with restriction, and two with a mixed pattern).

We found that the levels of thermophilic actinomycetes in floor dust were positively associated with any granulomatous disease-like symptoms (OR = 3.1 per IQR increase in exposure, Table 3). The association became stronger and remained significant (P-values <0.05) in models additionally adjusted for endotoxin or fungi, or similar in models adjusted for mycobacteria, total bacteria, or (1→3)-β-D-glucan (data not shown). The positive association also remained significant (P-value <0.05) when adjusting for all five exposures. The level of thermophilic actinomycetes was significantly negatively associated with ratio of FEV_1 (forced expiratory volume in 1 s) to FVC (forced vital capacity) by −2.8 per IQR (P = 0.02) and percent predicted average forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$) by −12.9% per IQR (P = 0.01), but not with percent predicted FEV_1. However, levels of thermophilic actinomycetes had a nonlinear relationship (P = 0.02) with percent predicted FVC, showing no effect at lower to medium levels and a positive association with higher levels within the range of the load of thermophilic actinomycetes in dust.

The level of non-tuberculous mycobacteria was positively marginally associated with Epi-asthma (OR = 1.5, P = 0.07; Table 3). The association became stronger and more significant (P-value <0.05) when endotoxin was added to the model and remained similar when other microbial agents were added to the model (data not shown). The positive association also remained significant (P-value <0.05) when adjusted for all five exposures simultaneously. In contrast, increasing levels of non-tuberculous mycobacteria were associated with increases in percent predicted $FEF_{25–75\%}$

Table 3 Odds ratios [per change in interquartile range (IQR) in exposure] of granulomatous disease (GD)- or asthma-like symptoms[a], or epidemiologically defined asthma for microbial measurements in dust from single environmental variable models adjusted for age, gender, smoking status (non-smoker/former vs. current smoker), and building tenure

	Microbial measurements, odds ratio per IQR increase (95% confidence interval)			
Health outcomes	Thermophilic actinomycetes	Non-tuberculuous mycobacteria	Total culturable bacteria	Endotoxin
Any GD-like symptoms in the last 4 weeks	3.1 (1.45–6.73)**	1.2 (0.80–1.93)	0.8 (0.55–1.29)	1.0 (0.55–1.86)
Shortness of breath on exertion	1.7 (0.81–3.71)	1.1 (0.68–1.73)	0.7 (0.44–1.24)	0.7 (0.37–1.40)
Fever and chills	1.0 (0.23–4.28)	1.5 (0.71–3.07)	1.0 (0.43–2.40)	1.2 (0.33–4.67)
Flu-like achiness	2.4 (1.10–5.07)**	1.3 (0.81–1.97)	0.8 (0.49–1.26)	1.1 (0.59–2.16)
Epidemiologically defined[b] asthma (Epi-asthma)	0.7 (0.33–1.65)	1.5 (0.97–2.43)*	0.8 (0.50–1.35)	0.6 (0.31–1.29)
Wheeze in the last 4 weeks	1.0 (0.47–2.16)	1.0 (0.64–1.66)	1.0 (0.61–1.57)	0.6 (0.30–1.21)
Shortness of breath in the last 4 weeks	2.0 (0.99–4.13)*	1.1 (0.71–1.69)	0.8 (0.52–1.27)	0.5 (0.28–1.06)*
Chest tightness in the last 4 weeks	1.4 (0.55–3.67)	1.2 (0.66–2.06)	0.8 (0.43–1.34)	0.7 (0.34–1.62)
Attack of cough	0.7 (0.32–1.54)	1.3 (0.83–2.08)	0.9 (0.58–1.50)	0.9 (0.49–1.83)
Epi-asthma or airway hyper-responsiveness[c]	0.5 (0.25–1.13)	1.3 (0.81–1.94)	0.9 (0.56–1.34)	0.6 (0.29–1.09)*

[a]Asthma-like symptoms included wheeze, attack of shortness of breath, chest tightness, attack of cough, or awakened by breathing difficulty.

[b]Epidemiologically defined asthma included occupants who reported three or more asthma-like symptoms in the last 4 weeks or physician-diagnosed current asthma.

[c]Nine more participants with airway hyper-responsiveness but who did not meet definition of epidemiologically defined asthma were added to the asthma outcome variable.

**P-value ≤0.05; *0.05 < P-value <0.1.

by 8.3% per IQR ($P = 0.02$), percent predicted FEV_1 by 4.5% per IQR ($P < 0.01$), and percent predicted FVC by 3.5% per IQR ($P = 0.02$), indicating a protective effect. The level of mycobacteria had a nonlinear relationship ($P = 0.049$) with percent predicted FVC, showing a positive association with lower levels and a negative association with higher levels.

The level of endotoxin was negatively but not significantly (OR = 0.6, $P > 0.1$) associated with Epi-asthma. When bronchial hyper-responsiveness was included in the asthma definition, the association became marginally significant (OR = 0.6, $P = 0.09$, Table 3). When the level of mycobacteria was added to the models, the protective effect of endotoxin on asthma became stronger and significant (ORs = 0.3–0.4, P-values <0.05) (data not shown). These negative associations of endotoxin with asthma remained significant (P-values <0.05) in models adjusted for all five exposures simultaneously. We did not find any evidence of nonlinear relationships (P-values >0.1) in these analyses. The level of endotoxin was marginally ($P = 0.08$) associated with increased percent predicted FEV_1 by 4.1% per IQR change in exposure, but not with FVC or FEV_1/FVC. Conversely, the levels of total culturable fungi and (1→3)-β-D-glucan in dust were not associated with any health symptoms, PFT results, or the number of work days missed due to respiratory illnesses.

Associations of respiratory health with microbial contaminants in heat pump drain tubes

A unit increase in the composite score of total bacteria (range: 2–19) in heat pump tubes was significantly associated with a decrease of about 20% in the odds of having asthma (Table 4). The potential protective effect was not changed by the addition of total fungi or (1→3)-β-D-glucan to the model, but became weaker and non-significant when the model was additionally adjusted for endotoxin (data not shown). The composite score of total bacteria was nonlinearly associated with an asthma definition met by either Epi-asthma or airway hyper-responsiveness ($P = 0.03$), showing quadratic relationships with an adverse effect at lower levels but a protective effect at higher levels. When we modeled 37 occupants who participated in spirometry testing and had been potentially exposed to contaminants in heat pump drain tubes, the score of total bacteria was positively associated with FEV_1/FVC (0.44, $P = 0.095$) and percent predicted FEV_1 (1.04%, $P = 0.04$).

The composite score of endotoxin (range: 2–19) in heat pumps was also significantly associated with decreased odds of two or more granulomatous disease-like symptoms or asthma by 20% (Table 4). These associations were not affected by total bacteria, total fungi, or (1→3)-β-D-glucan. The composite score of endotoxin and asthma had marginally significant (P-values = 0.06) nonlinear (cubic) relationships, showing adverse effect at lower or higher levels and protective effect at the mid-range levels. However, the composite score of endotoxin was not associated with any PFT results.

The composite scores of total bacteria and endotoxin in the heat pump condensate drains had a significant protective effect on the number of work days missed due to respiratory illnesses in the last 12 months (both coefficients = −0.18 days per unit composite score, P-values = 0.01). However, composite scores of total culturable fungi and (1→3)-β-D-glucan in heat pump systems were not associated with any health symptoms, PFT results, or the number of work days missed due to respiratory illnesses.

Table 4 Odds ratios [per one composite score increase in exposure] of granulomatous disease (GD)- or asthma-like symptoms[a], or epidemiologically defined asthma for microbial measurements in heat pump drain tube from single environmental variable models adjusted for age, gender, smoking status (non-smoker/ever smoker), and building tenure

	Microbial measurements, odds ratio (95% confidence interval)	
Health outcomes	Total culturable bacteria	Endotoxin
Two or more GD-like symptoms in the last 4 weeks	0.8 (0.65–1.01)*	0.8 (0.67–0.98)**
Shortness of breath on exertion	0.9 (0.78–1.03)	0.9 (0.79–1.04)
Fever and chills	0.9 (0.72–1.19)	0.8 (0.64–1.12)
Flu-like achiness	0.9 (0.78–1.02)	0.9 (0.76–0.99)**
Epidemiologically defined[b] asthma	0.8 (0.71–1.00)**	0.8 (0.67–0.96)**
Wheeze in the last 4 weeks	0.9 (0.81–1.07)	0.9 (0.76–1.01)*
Shortness of breath in the last 4 weeks	0.9 (0.77–1.03)	0.9 (0.80–1.05)
Chest tightness in the last 4 weeks	1.0 (0.81–1.13)	0.9 (0.72–1.01)*
Attack of cough	1.0 (0.87–1.13)	0.9 (0.79–1.03)
Epidemiologically defined asthma[b] or airway hyper-responsiveness[c]	0.8 (0.72–0.96)**	0.8 (0.72–0.95)**

[a]Asthma-like symptoms included wheeze, attack of shortness of breath, chest tightness, attack of cough, or awakened by breathing difficulty.

[b]Epidemiologically defined asthma included occupants who reported three or more asthma-like symptoms in the last 4 weeks or physician-diagnosed current asthma.

[c]Nine more participants with airway hyper-responsiveness but who did not meet definition of epidemiologically defined asthma were added to the asthma outcome variable.

**P-value ≤0.05; *0.05 < P-value <0.1.

Discussion

We found diverse fungal and bacterial flora including actinomycetes and non-tuberculous mycobacteria, in the floor dust of the study building. Growth of actinomycetes requires high moisture content (water activity >0.9) (Rintala, 2011; World Health Organization, 2009), and their occurrence in indoor environments may indicate water damage (Meklin et al., 2003; Nevalainen et al., 1991; Samson et al., 1994). The load of endotoxin in dust was more than two times greater (28,000 vs. 12,000 EU/m^2) than that reported in another water-damaged office building study (Cho et al., 2011; Park et al., 2008), indicating potential heavy contamination with Gram-negative bacteria. Gram-negative bacteria also require high water content for growth (Otten and Burge, 1999). *Penicillium chrysogenum* and *Cladosporium sphaerospermum* were the most prevalent fungi in dust, and *Cladosporium sphaerospermum* is one of the fungal species that were reportedly indicative of moisture damage in houses (Lignell et al., 2008). Abundance of these bacteria and fungi supports the previous report of a long history of water incursion in the building (Laney et al., 2009).

We found that increasing thermophilic actinomycetes levels in floor dust were significantly associated with decreased pulmonary function and increased odds of having symptoms reflecting possible granulomatous disease. Prevalences of the three granulomatous disease-like symptoms among the occupants were similar to those reported in another study of a large office building with eight hypersensitivity pneumonitis and six sarcoidosis cases, a long history of moisture incursions, and high fungal and bacterial contamination (Cox-Ganser et al., 2005; Park et al., 2006). Actinomycetes were cultured from the majority of dust samples in the building, but the level (GM = 850 CFU/g) was considerably lower than that reported from a study of farm and non-farm houses (median = 9000 CFU/g) (Kärkkäinen et al., 2010). Our findings suggest that exposure to actinomycetes may be an important contributor to granulomatous disease-like symptoms that were prevalent in these occupants.

We previously reported six physician-diagnosed sarcoidosis cases (three former and three current employees) but no physician-diagnosed HP cases among occupants (Laney et al., 2009). Both sarcoidosis of the lung and HP are granulomatous lung diseases but considered distinct clinical entities, although they may not be easily differentiated clinically and pathologically (Newman and Newman, 2012). The disease may be diagnosed as sarcoidosis when the etiology is unknown, but as HP when etiologic agents are identified. With only six cases, we were not able to analyze associations with exposure to thermophilic actinomycetes or other microbial agents. However, we found that the workstations of five sarcoidosis cases (one case's workstation was unknown) were positive for culturable thermophilic actinomycetes. Therefore, considering that the prevalences of symptoms reflecting possible granulomatous disease were relatively high in this population, there might be undiagnosed cases of sarcoidosis or HP, or those symptomatic occupants may have subclinical conditions of these diseases.

The newly proposed class name, Actinobacteria, is gradually replacing the term 'actinomycetes' (Stackebrandt and Schumann, 2006). The Actinobacteria now include more than 230 genera of Gram-positive bacteria (Rintala, 2011). We identified actinomycetes or eight different genera (*Arthrobacter, Brevibacterium*, *Cellulomonas*, *Kocuria*, *Microbacterium*, *Micrococcus*, *Rhodococcus,* and *Mycobacterium*) in dust that belong to the Actinobacteria class. In this building, 88% of samples were positive for actinomycetes, mycobacteria, or the top three bacterial genera belonging to Actinobacteria. Our findings show that Gram-positive bacteria, especially Actinobacteria, were the dominant microbial flora in dust, implying that this group of bacteria might be partly responsible for various health effects in these occupants.

HP has been recognized among various groups of people, including people that used hot tubs, workers handling machining fluids, and lifeguards at indoor swimming pools. In some of those instances, *Mycobacterium* species isolated from environmental samples were implicated as a causative agent (Falkinham, 2003). In a recent publication from the GABRIEL Advanced survey, Valkonen et al. (2015) reported that levels of *Mycobacterium* species in mattress dust from farm and control children measured using a quantitative PCR method were negatively associated with prevalence of atopy in children. However, exposure to non-tuberculous mycobacteria in damp buildings in relation to occupants' health is not well understood. Culturable mycobacteria were isolated in 23% of 88 building material samples from water-damaged buildings (range: 60–2.0 $\times$ 10^7 CFU/g) (Torvinen, 2006). High concentrations (10^6 CFU/g) of mycobacteria were found in gypsum board with water damage in a children's daycare center (Andersson et al., 1997). Using the mycobacteria isolated from moldy buildings, Huttunen et al. (2000) showed that exposure to mycobacteria increased production of cytokines (IL-6 and TNF-α), nitric oxide (NO), and reactive oxygen species in murine macrophages in a dose-dependent way. They also reported that mycobacterial exposure induced inflammatory pathways in human lung epithelial cells and macrophages (Huttunen et al., 2001). *Mycobacterium terrae* isolated from air in a water-damaged building also produced inflammation in mouse lungs (Jussila et al., 2002). Falkinham suggested that exposure to mycobacteria or their metabolites in water-damaged buildings may result in airway

inflammatory illnesses in occupants (Falkinham, 2003). In our study, we found that 19% of floor dust samples were positive for mycobacteria and that mycobacteria in floor dust were significantly associated with asthma or asthma symptoms. Thus, we cannot rule out the possibility that exposure to non-tuberculous mycobacteria in water-damaged buildings may exacerbate asthma by inducing airway inflammation.

We found no evidence of associations between respiratory illnesses and fungal exposure, which is contrary to a number of research findings (Cox-Ganser et al., 2005; Park et al., 2008; World Health Organization, 2009). Huttunen et al. (2003) studied inflammatory and cytotoxic effects of individual exposure to three bacterial (*Streptomyces californicus*, *Bacillus cereus*, and *Pseudomonas fluorescens*) and three fungal species (*Aspergillus versicolor*, *Penicillium spinulosum*, and *Stachybotrys chartarum*) using murine and human cell lines. They reported that bacteria [*Streptomyces californicus* (Actinobacteria) and *Pseudomonas fluorescens* (Gram-negative bacteria)] were stronger inducers of inflammation than fungi in both types of cell lines. Our findings of no associations between respiratory illnesses and fungal exposure or protective effects of endotoxin or bacterial exposure on health may be explained by the complex health mechanisms due to simultaneous exposure to various bacteria and fungi (Karvonen et al., 2014). Interactions among them may produce antagonistic, additive, or synergistic effects (Huttunen et al., 2004; Park et al., 2006; Rintala, 2011), depending on microbial diversity and concentration. These health outcomes may become even more complicated by difference in individual susceptibility among exposed people. Our study findings may imply that the extent, frequency, and duration of water damage, wet and dry cycles, or type of building materials create building-specific environmental conditions favorable for unique microbiota. The microbiota in individual buildings may present unique inflammatory and cytotoxic potential, which could result in different health profiles among buildings.

During the cooling season (or summer), the drain pans and tubes collect condensate water from the heat pumps, which is drained into the main drainpipe. However, if condensate water is trapped in the drain pans or tubes, the water may serve as a reservoir for potential microbial growth. The heat pump drain system in the annex of the study building was constructed with flexible plastic drain tubes that did not have antibacterial properties and the tubes had condensation water and dirt inside that supported microbial growth. If condensate in the drain pans is regularly cleaned and properly treated and the drain pipes are constructed with copper that is known to have antibacterial properties (Grass et al., 2011), the condensate drain systems in the heat pumps may be better protected from microbial contamination. The contaminated condensate water with dirt in the traps can dry out, which may produce air passages through the tubes between the heat pumps and drain system. Via tracer gas testing and pressure mapping, we found several airflow patterns existed within the drainage system that may have allowed microbial contaminants to be potentially dispersed into occupied spaces within the facility (Turner Building Science & Design, unpublished data, 2007). Thus, we cannot rule out the possibility that improperly managed condensate in drain systems in the heat pumps may be a source of exposure to microbial contaminants from the HVAC system.

The culture method used in the study has limitations as only a small proportion of microbial flora in the samples is cultivable on selected media (Park et al., 2008; Toivola et al., 2002; Torvinen et al., 2010). However, studies comparing culture and quantitative polymerase chain reaction methods showed that dominant fungal taxa in dust were consistent between the two methods and that the methods were significantly correlated (Lignell et al., 2008; Pitkaranta et al., 2011). Thus, our study findings were not likely to be by chance. In our study, only 19% of dust samples were positive for culturable mycobacteria. This low prevalence might be influenced by overgrowth of other bacteria during the long incubation period. However, because we used selective growth media inoculated with four serial dilutions of dust suspension containing a fungal suppressant, underestimation of occurrence might have been minimized. We assigned a value of LOD/2 to the non-detectable samples for statistical analyses. In doing this, we might have produced non-differential error in exposure to non-tuberculous mycobacteria, resulting in potential underestimation of ORs. During the building diagnostic survey, we found potential exposure to oil soot in occupants that resulted from the boiler breaching (Turner Building Science & Design, unpublished data, 2007). But we had no systematic exposure measurement data and thus could not evaluate any potential effect of soot exposure on the associations we found in the study. Our environmental sampling was conducted approximately 9 months after the health survey, which may not have captured the true exposure of occupants at the time of the survey. However, there had been no remediation or significant changes in the environment of the building before or after the building was vacated. Therefore, our environmental results should still reasonably represent prior occupants' earlier exposure.

Conclusions

Thermophilic actinomycetes and mycobacteria were significantly associated with decreased lung function, as well as respiratory and/or systemic symptoms

reflecting possible granulomatous disease or asthma. In contrast, total bacteria and endotoxin were negatively associated, and fungi were not associated with health outcomes. These mixed findings imply that individual buildings may have a unique occupant health profile associated with environmental microbiota that are distinctively created by the sources, frequency, duration, and extent of water damage, type of building materials, and patterns of wet and dry cycles. Our study findings also suggest that bacteria, including actinomycetes and mycobacteria, should be considered important exposures in epidemiologic studies of water-damaged buildings.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Supporting Information

Additional Supporting Information may be found in the online version of this article:
Table S1. Fungal species identified in floor dust and liquid and sludge of heat pump system.

References

Andersson, M.A., Nikulin, M., Koljalg, U., Andersson, M.C., Rainey, F., Reijula, K., Hintikka, E.L. and Salkinoja-Salonen, M. (1997) Bacteria, molds, and toxins in water-damaged building materials, *Appl. Environ. Microbiol.*, **63**, 387–393.

Cho, S.J., Park, J.H., Kreiss, K. and Cox-Ganser, J.M. (2011) Levels of microbial agents in floor dust during remediation of a water-damaged office building, *Indoor Air*, **21**, 417–426.

Cox-Ganser, J.M., White, S., Jones, R., Hilsbos, K., Storey, E., Enright, P.L., Rao, C. and Kreiss, K. (2005) Respiratory morbidity in office workers in a water-damaged building, *Environ. Health Perspect.*, **113**, 485–490.

Falkinham, J.O. (2003) Mycobacterial aerosols and respiratory disease, *Emerg. Infect. Dis.*, **9**, 763–767.

Grass, G., Rensing, C. and Solioz, M. (2011) Metallic copper as an antimicrobial surface, *Appl. Environ. Microbiol.*, **77**, 1541–1547.

Huttunen, K., Ruotsalainen, M., Iivanainen, E., Torkko, P., Katila, M. and Hirvonen, M. (2000) Inflammatory responses in RAW264.7 macrophages caused by mycobacteria isolated from moldy houses, *Environ. Toxicol. Pharmacol.*, **8**, 237–244.

Huttunen, K., Jussila, J., Hirvonen, M.R., Iivanainen, E. and Katila, M.L. (2001) Comparison of mycobacteria-induced cytotoxicity and inflammatory responses in human and mouse cell lines, *Inhal. Toxicol.*, **13**, 977–991.

Huttunen, K., Hyvarinen, A., Nevalainen, A., Komulainen, H. and Hirvonen, M.R. (2003) Production of proinflammatory mediators by indoor air bacteria and fungal spores in mouse and human cell lines, *Environ. Health Perspect.*, **111**, 85–92.

Huttunen, K., Pelkonen, J., Nielsen, K.F., Nuutinen, U., Jussila, J. and Hirvonen, M.R. (2004) Synergistic interaction in simultaneous exposure to *Streptomyces californicus* and *Stachybotrys chartarum*, *Environ. Health Perspect.*, **112**, 659–665.

Jussila, J., Komulainen, H., Huttunen, K., Roponen, M., Iivanainen, E., Torkko, P., Kosma, V.M., Pelkonen, J. and Hirvonen, M.R. (2002) *Mycobacterium terrae* isolated from indoor air of a moisture-damaged building induces sustained biphasic inflammatory response in mouse lungs, *Environ. Health Perspect.*, **110**, 1119–1125.

Kärkkäinen, P.M., Valkonen, M., Hyvarinen, A., Nevalainen, A. and Rintala, H. (2010) Determination of bacterial load in house dust using qPCR, chemical markers and culture, *J. Environ. Monit.*, **12**, 759–768.

Karvonen, A.M., Hyvarinen, A., Rintala, H., Korppi, M., Taubel, M., Doekes, G., Gehring, U., Renz, H., Pfefferle, P.I., Genuneit, J., Keski-Nisula, L., Remes, S., Lampi, J., von Mutius, E. and Pekkanen, J. (2014) Quantity and diversity of environmental microbial exposure and development of asthma: a birth cohort study, *Allergy*, **69**, 1092–1101.

Lacey, J. and Crook, B. (1988) Fungal and actinomycete spores as pollutants of the workplace and occupational allergens, *Ann. Occup. Hyg.*, **32**, 515–533.

Laney, A.S., Cragin, L.A., Blevins, L.Z., Sumner, A.D., Cox-Ganser, J.M., Kreiss, K., Moffatt, S.G. and Lohff, C.J. (2009) Sarcoidosis, asthma, and asthma-like symptoms among occupants of a historically water-damaged office building, *Indoor Air*, **19**, 83–90.

Lignell, U., Meklin, T., Rintala, H., Hyvarinen, A., Vepsalainen, A., Pekkanen, J. and Nevalainen, A. (2008) Evaluation of quantitative PCR and culture methods for detection of house dust fungi and streptomycetes in relation to moisture damage of the house, *Lett. Appl. Microbiol.*, **47**, 303–308.

Meklin, T., Hyvarinen, A., Toivola, M., Reponen, T., Koponen, V., Husman, T., Taskinen, T., Korppi, M. and Nevalainen, A. (2003) Effect of building frame and moisture damage on microbiological indoor air quality in school buildings, *AIHA J.*, **64**, 108–116.

Milton, D.K., Feldman, H.A., Neuberg, D.S., Bruckner, R.J. and Greaves, I.A. (1992) Environmental endotoxin measurement: the kinetic Limulus assay with resistant-parallel-line estimation, *Environ. Res.*, **57**, 212–230.

Nevalainen, A. and Seuri, M. (2005) Of microbes and men, *Indoor Air*, **15**, 58–64.

Nevalainen, A., Pasanen, A., Niininen, M., Reponen, T., Kalliokoski, P. and Jantunen, M. (1991) The indoor air quality in Finnish homes with mold problems, *Environ. Int.*, **17**, 299–302.

Newman, K.L. and Newman, L.S. (2012) Occupational causes of sarcoidosis, *Curr. Opin. Allergy Clin. Immunol.*, **12**, 145–150.

Otten, J. and Burge, H. (1999) Bacteria. In: Macher, J. (ed.) *Bioaerosols*, Cincinnati, OH, American Conference of Governmental Industrial Hygienists, 1–10.

Park, J.H., Cox-Ganser, J.M., Rao, C.Y. and Kreiss, K. (2006) Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building, *Indoor Air*, **16**, 192–203.

Park, J.H., Cox-Ganser, J.M., Kreiss, K., White, S.K. and Rao, C.Y. (2008) Hydrophilic fungi and ergosterol associated with respiratory illness in a water-damaged building, *Environ. Health Perspect.*, **116**, 45–50.

Pitkaranta, M., Meklin, T., Hyvarinen, A., Nevalainen, A., Paulin, L., Auvinen, P., Lignell, U. and Rintala, H. (2011) Molecular profiling of fungal communities in moisture damaged buildings before and after remediation–a comparison of culture-dependent and culture-independent methods, *BMC Microbiol.*, **11**, 235.

Rintala, H. (2011) Actinobacteria in indoor environments: exposures and respiratory

health effects, *Front. Biosci. (Schol. Ed.)*, **3**, 1273–1284.
Rintala, H., Pitkaranta, M., Toivola, M., Paulin, L. and Nevalainen, A. (2008) Diversity and seasonal dynamics of bacterial community in indoor environment, *BMC Microbiol.*, **8**, 1–13.
Samson, R., Flannigan, B., Flannigan, M., Adan, O. and Hoekstra, E. (1994) Recommendations, In: Samson, R. and Flannigan, B. (eds) *Health Implications of Fungi in Indoor Environments*, Amsterdam, the Netherlands, Elsevier Science, 529–538.
Shogren, E.S. and Park, J.H. (2011) Presampling contamination of filters used in measurements of airborne (1 –> 3)-beta-D-glucan based on glucan-specific Limulus amebocyte lysate assay, *J. Environ. Monit.*, **13**, 1082–1087.
Stackebrandt, E. and Schumann, P. (2006) Introduction to the taxonomy of actinobacteria. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. (eds) *The Prokaryotes*, New York, NY, Springer-Verlag, 297–321.
Taubel, M., Rintala, H., Pitkaranta, M., Paulin, L., Laitinen, S., Pekkanen, J., Hyvarinen, A. and Nevalainen, A. (2009) The occupant as a source of house dust bacteria, *J. Allergy Clin. Immunol.*, **124**, 834–840.
Toivola, M., Alm, S., Reponen, T., Kolari, S. and Nevalainen, A. (2002) Personal exposures and microenvironmental concentrations of particles and bioaerosols, *J. Environ. Monit.*, **4**, 166–174.
Torvinen, E.E.M. (2006) Mycobacteria and fungi in moisture-damaged building materials, *Appl. Environ. Microbiol.*, **72**, 6822–6824.
Torvinen, E., Torkko, P. and Rintala, A.N. (2010) Real-time PCR detection of environmental mycobacteria in house dust, *J. Microbiol. Methods*, **82**, 78–84.
Valkonen, M., Wouters, I.M., Täubel, M., Rintala, H., Lenters, V., Vasara, R., Genunelt, J., Braun-Fahrländer, C., Piarroux, R., von Mutius, E., Heederik, D. and Hyvärinen, A. (2015) Bacterial exposures and associations with atopy and asthma in children, *PLoS One*, **10**, e0131594.
World Health Organization (2009) Health effects associated with dampness and mould. In: Heseltine, E. and Rosen, J. (eds) *WHO Guidelines for Indoor Air Quality: Dampness and Mould*, Copenhagen, Denmark, WHO Regional Office for Europe, 63–92.