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Streptomycetes in house dust: associations with housing characteristics and endotoxin

Abstract In addition to mold, indoor bioaerosols also contain bacterial components that may have implications for human health. Endotoxin is a cell wall component in Gram-negative bacteria present at varying levels indoors that has been found to have respiratory health implications. *Streptomyces* is a large genus of Gram-positive bacteria, and some species have been shown to produce inflammatory reactions *in vitro* and *in vivo*. The aim of this study was to determine predictors of streptomycetes levels in house dust and to compare the variation in streptomycetes levels with that in endotoxin levels. Dust was collected by floor vacuuming from 178 homes in the Cincinnati metropolitan area. Streptomycetes levels were measured by quantitative PCR, and endotoxin was assayed by the *Limulus* amebocyte lysate method. Associations between home characteristics and bacterial contaminants, expressed as concentration and load, were investigated through multiple regression analyses. The presence of two or more dogs was a strong predictor of both streptomycetes and endotoxin levels. Season of dust collection and levels of outdoor molds were predictors of streptomycetes but not endotoxin levels. In contrast, number of inhabitants was a significant predictor of endotoxin load only. Neither streptomycetes nor endotoxin levels were associated with metrics of moisture damage.

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Practical Implications
This study adds to the understanding of the sources of bacterial contaminant in indoor environments. The results suggest that streptomycetes have mostly outdoor sources, whereas indoor sources are more important for endotoxin. The results also indicate that the presence of pets, particularly two or more dogs, is a strong source of bacterial contamination. In this study, neither streptomycetes nor endotoxin levels were significantly associated with metrics for moisture damage. Both endotoxin and streptomycetes levels may represent too large and diverse bacterial taxa to be consistent indicators of indoor moisture damage.

Introduction

Airborne microbial contaminants in the indoor environment have been shown to be associated with respiratory health outcomes in children and adults (Sahakian et al., 2008). Building dampness can cause mold growth, and the association between mold exposure and asthma is well established (Vesper et al., 2006; WHO, 2009). Indoor air also contains components of bacterial origin, such as spores, cell fragments, cell wall components, and toxins. A number of Gram-positive and Gram-negative bacteria species have been isolated from moisture-damaged building materials (Andersson et al., 1997; Peltola et al., 2001; Suihko et al., 2009; Torvinen et al., 2006), and a large variety of bacteria have also been detected in settled dust from both moisture-damaged and normal buildings (Andersson et al., 1997; Rintala et al., 2008). Bacteria found in indoor dust and aerosols have both indoor sources, such as humans and pets, and outdoor sources, such as soil, but the precise sources and means of transportation and dispersion for different species and genera are still not well understood. The health effects of exposure to airborne components of bacterial origin have been less studied than those of exposure to mold. It has, however, long been recognized that inhalation exposure to nonpathogenic bacteria and their components can result in atopic disease, including asthma, as well as nonatopic inflammatory diseases (Douwes et al., 2003). Inverse associations between

bacterial exposures and atopic diseases have also been reported, in accordance with the so-called hygiene hypothesis (Douwes et al., 2006; Maier et al., 2010; Sordillo et al., 2010).

Endotoxin is the biologically active lipid A moiety of lipopolysaccharide (LPS) structures found in the cell wall of Gram-negative bacteria. Both epidemiological and animal studies have implicated endotoxin in the development of atopy (Simpson and Martinez, 2010). Endotoxin is universally found in indoor dust, but the levels can vary considerably. Home characteristics usually found to be associated with endotoxin levels in dust include presence of dogs, presence of cats, age of building, and type of room where sampling was carried out (Abraham et al., 2005; Bischof et al., 2002; Gereda et al., 2001; Park et al., 2000; Thorne et al., 2009; Wickens et al., 2003). Most studies have failed to detect an association between endotoxin levels and localized moisture damage, although Thorne et al. (2009) found a significant association between dust endotoxin and observed mold growth.

Endotoxin represents only part of the total exposure to bacteria. Gram-positive bacteria, which lack LPS, are also found in indoor environments. Several genera belonging to the Gram-positive class Actinobacteria have received attention as contaminants in moisture-damaged buildings with possible implications for respiratory health. Some Actinobacteria genera commonly isolated from moisture-damaged buildings are *Streptomyces* (Andersson et al., 1997), *Mycobacterium* (Torvinen et al., 2006), and *Nocardiopsis* (Peltola et al., 2001). *Streptomyces* is a large genus of Gram-positive, spore forming, soil bacteria that belongs to the Actinobacteria class. Several *Streptomyces* strains have been shown to be potent inducers of inflammatory reactions *in vitro* (Huttunen et al., 2003; Jussila et al., 1999) and *in vivo* (Jussila et al., 2003). Streptomycetes are believed to thrive in moisture-rich environments, and a number of *Streptomyces* species, e.g., *Streptomyces griseus* and *S. coelicolor*, have been isolated from moisture-damaged building materials (Rintala et al., 2002; Suihko et al., 2009).

While indoor endotoxin exposure has been well characterized, less is known about sources and predictors of Gram-positive bacterial contaminants in indoor environments. Because many Actinobacteria, including *Streptomyces* species, are ubiquitous soil dwellers, it can be expected that Actinobacteria found in indoor air and dust have outdoor sources in addition to possible sources related to moisture damage. Very few studies, however, have investigated Actinobacteria concentrations in indoor environments, and there have been no previous studies of associations between levels of indoor Actinobacteria and home characteristics. In this investigation, we measured the concentrations and loads of streptomycetes in indoor floor dust for 178 Midwestern US homes using quantitative PCR. We examined the data for associations with a number of home characteristics, including presence of pets, number of inhabitants, moisture damage, presence of mold, and general home characteristics such as age of building and household income. In addition, we wanted to determine which sources were in common and which sources were different for streptomycetes and the more frequently studied contaminant endotoxin. It has been suggested that endotoxin may be a sentinel marker of both Gram-positive and Gram-negative bacterial exposures arising from shared sources, and these exposures may influence immune responses through shared pathways (Sordillo et al., 2010). Therefore, we also measured levels of endotoxin and compared the results with those for streptomycetes.

Materials and methods

Homes in the cohort

Dust samples and information about home characteristics were obtained from the homes of 178 children that participated in the Cincinnati Childhood Asthma and Air Pollution Study (CCAAPS), a birth cohort study of 777 children (LeMasters et al., 2006; Reponen et al., 2010). Infants living in the Cincinnati (Ohio) and Northern Kentucky metropolitan area were identified through birth records and enrolled in the CCAAPS at approximately 6–7 months of age. Based on the CCAAPS year 1 home inspection (Cho et al., 2006), 50% of the homes selected for the study were homes previously classified as high-mold, and 50% were previously classified as low-mold. The dust sampling and assessment of homes for this study were performed when the children were 7 years old. The study was approved by the University of Cincinnati Institutional Review Board, and informed consent was obtained by a parent at the time of each home visit.

Home visits

Selected homes were visited by trained two-person teams who administered a detailed questionnaire, performed dust sampling as described below, and inspected the home for signs of moisture damage and/or mold growth. The sampling was conducted in the room where the enrolled child spent most of his/her daytime, which is referred to as the primary activity room (PAR). The questionnaire included questions about the presence of pets, number of inhabitants, factors related to the indoor climate, such as use of humidifier and central air conditioning, and general home characteristics, such as age of building, history of moisture damage, and household income. All rooms in the home were inspected for signs of visible moisture damage or mold growth, and any discovered damage

was measured and photographed. The inspection team also measured the temperature and relative humidity in the PAR and noted any moldy odor in the house. Based on the questionnaire and home inspections, the homes were categorized into three classes with respect to the degree of water damage and mold, as described by Cho et al. (2006). Class 0 homes had no history or signs of mold/water damage, including no moldy odor. Class 1 homes had either minor indications of mold/water damage or a history thereof. Class 2 homes had at least 0.2 m^2 of visible mold, or mold and water damage combined.

Indoor dust sampling and dust endotoxin analysis

Dust sampling was performed as previously described (Cho et al., 2006). Briefly, for carpeted floors, a 2-m^2 area was vacuumed at a rate of 2 min/m^2, and for hardwood floors, dust was collected from an entire room at 1 min/m^2. Collected dust was sieved through a 355-μm sieve, and the resulting fine dust was stored at −20°C until further use. Determination of endotoxin concentrations in dust was performed according to the *Limulus* amebocyte lysate method (Milton et al., 1997; Campo et al., 2006), using the Pyrochrome® Endotoxin Detection Reagent Kit from Associates of Cape Cod, Inc. (East Falmouth, MA, USA) according to manufacturer's instructions. Twenty-five micrograms of dust were suspended in 1 ml of pyrogen-free water and sonicated 1 h with vortexing for 15 s every 15 min. Samples were centrifuged 1 min at 5200 *g*, and the supernatant was used for the assay. Endotoxin concentration in dust was expressed as endotoxin units (EU) per microgram of the collected dust, and endotoxin load was expressed as EU per m^2 floor area. Load was calculated by multiplying concentration with total amount of dust collected and dividing with m^2 floor area vacuumed.

DNA extractions and quantitative PCR analysis of streptomycetes

Five milligrams of fine dust was added to sterile 2-ml tubes containing 0.3 g of acid-washed glass beads (#G1277; Sigma-Aldrich, St Louis, MO, USA). To the tubes were also added 0.35 ml Lysis buffer (GeneRite, North Brunswick, NJ, USA), together with 10 μl of a 2×10^8 conidia/ml reference suspension of *Geotrichum candidum* (#7863, University of Alberta Microfungus Collection and Herbarium), which was included as an internal positive control. The tubes were shaken in a Mini Bead-Beater (Biospec Products, Bartlesville, OH, USA) for 1 min at a maximum speed, and DNA was isolated using DNA-EZ kit from GeneRite (North Brunswick, NJ, USA) according to manufacturer's instructions.

Amounts of streptomycetes and *Geotrichum candidum* cells were assayed by quantitative PCR (qPCR) in separate reactions using the Roche LightCycler® 480 System (Roche Applied Science, Indianapolis, IN, USA). Primers and probe targeted toward the ribosomal 23S gene and specific for the *Streptomyces* genus have been described previously (Rintala and Nevalainen, 2006). Each reaction contained 1 × Taqman® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 1 μM of each primer, 80 nM of the probe, 0.01 mg/ml BSA, and 5 μl DNA in a total reaction volume of 25 μl. The PCR program for both primer sets consisted of an initial incubation step at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10 s and annealing/extension at 60°C for 30 s. All reactions were carried out in duplicate. For each sample, the average Ct number for the *Geotrichum candidum* analysis was subtracted from the average Ct number for the streptomycetes analysis.

For the standard curve, DNA was extracted from a reference suspension of *Streptomyces anulatus* spores (ATCC type strain #27416 from ATCC, Manassas, VA, USA) as described above. Serial dilutions were prepared and analyzed by qPCR as described above. The average *Geotrichum candidum* Ct number for the undiluted DNA was subtracted from the Ct numbers for the streptomycetes serial dilutions, and the resulting numbers were plotted against the spore concentrations for each dilution. The resulting standard curve was linear, with $r^2 = 0.998$.

Streptomycetes concentration was expressed as cells per microgram dust, and streptomycetes load was expressed as cells per m^2 floor area. Load was derived from concentration as described above for endotoxin.

Measurement of Environmental Relative Moldiness Index (ERMI)

Environmental Relative Moldiness Index (ERMI) was developed to quantify levels of mold species specifically associated with moisture-damaged homes and has been shown to be associated with respiratory illnesses in children (Vesper et al., 2004, 2007). The method is based on quantitative PCR analysis of 26 molds (Group 1 molds) associated with building water damage and 10 ubiquitous molds not associated with water damage (Group 2 molds) (Vesper, 2010). The index is calculated by subtracting the sum of log-transformed Group 2 mold concentrations (SLG2) from the sum of log-transformed Group 1 concentrations (SLG1). The measurement of ERMI for this study was taken on DNA isolated from the dust samples. The quantitative PCR analyses were performed as described in detail earlier (Haugland et al., 2004).

Statistical methods

Preliminary analyses were performed to assess outcome distributions. The four outcomes evaluated were streptomycetes concentration, streptomycetes load, endotoxin

concentration, and endotoxin load. The association between $\log_e$-transformed values of each outcome and each home characteristic (independent variable) was evaluated by univariate regression. The assumption of linearity of continuous and ordinal home characteristics was evaluated by graphs and model fit (Akaike Information Criteria). Linearly modeled home characteristics included number of inhabitants, humidifier use, temperature in PAR, relative humidity in PAR, the year the home was built, ERMI, and SLG2. The remaining home characteristics were modeled categorically. Model building was performed separately for each outcome. Final multivariate models were obtained by sequential analyses using backward stepwise regression with a forward approach. All independent variables that were univariately significant ($P < 0.20$) were included in an initial multiple linear regression model. Variables were assessed for removal one at a time, beginning with the variable having the highest *P*-value. Previously removed independent variables were re-entered at various stages to allow for the possibility that significance levels may have changed because of changes in the model. The final multivariate model included all independent variables having $P < 0.05$ and one independent variable with $P > 0$ .05. Ratios of the antilogs of predicted mean values, corresponding to a prespecified difference in levels of each home characteristic, were calculated to interpret the effect of parameter estimates on the original scale.

Results

Distributions of streptomycetes and endotoxin levels in floor dust

The distributions of the investigated home characteristics are presented in Table 1, along with the unadjusted geometric means for the four outcome variables: streptomycetes concentration, streptomycetes load, endotoxin concentration, and endotoxin load. The geometric mean of streptomycetes concentration in dust for all homes sampled ($n = 178$) was 1655 cells/mg, and the geometric mean of streptomycetes load was 7.28×10^5 cells/m^2. Both streptomycetes concentration and load were significantly lower for samples collected in the summer than for samples collected in the other seasons ($P < 0.01$). Presence of cats was significantly associated with higher streptomycetes concentrations, but not loads. Interestingly, whereas the presence of one dog was not associated with altered streptomycetes levels, the presence of two or more dogs was strongly associated with increases in both concentrations and loads ($P < 0.01$). Increases in relative humidity as well as temperature were both associated with significantly lower streptomycetes concentrations and loads.

The geometric mean of endotoxin concentration in dust for all 178 homes was 160 EU/mg, and the geometric mean of endotoxin load was 7.1×10^4 EU/m^2. Similar to streptomycetes, both endotoxin concentration and load were strongly associated with the presence of two or more dogs, whereas there was no difference in endotoxin levels between homes with one dog and homes with no dogs. Also similar to streptomycetes, the presence of cats was associated with higher endotoxin concentration, but not with endotoxin load. Number of inhabitants was significantly associated with endotoxin load, whereas there was a trend for endotoxin concentration ($P = 0.1$). Household income was borderline significantly associated with endotoxin load ($P = 0.06$), with income < \$40 000/year associated with higher load.

Correlations between outcome variables

For both streptomycetes and endotoxin, concentration was significantly correlated with load ($P < 0.01$). As shown in Figure 1, the Spearman product moment correlation coefficient between log-transformed streptomycetes concentration and load, $r = 0.64$, was very close to the correlation coefficient between endotoxin concentration and load, which was 0.67. The correlation between streptomycetes concentration and endotoxin concentration was weak, with $r = 0.26$ ($P < 0.01$). The correlation between streptomycetes load and endotoxin load, however, was much higher ($r = 0.72$; $P < 0.01$).

Multiple linear regression models for determinants of streptomycetes and endotoxin levels in dust

Adjusted means ratios and 95% confidence intervals for multiple linear regression models of all four outcomes are presented in Table 2. Home characteristics that were significantly associated with an outcome variable are included in the table, along with the nonsignificant home characteristic with the lowest *P*-value. Season of dust collection remained in the models as a strong determinant of both streptomycetes concentration and load in dust, with dust collected in the summer having considerably lower streptomycetes levels than dust collected in the other seasons ($P < 0.01$). The presence of dogs was also a strong predictor of streptomycetes levels, with mean concentration being 2.32 higher in homes with two or more dogs than in homes with less than two dogs. Chi-square analysis showed that there was no significant association between income and presence of two or more dogs (data not shown). The corresponding ratio for streptomycetes load was 2.79. Whereas the association of streptomycetes levels with ERMI was only a trend and not statistically significant, the association with SLG2 was greater and clearly significant for both concentration and load. A few home characteristics were associated with streptomycetes levels in one measurement unit

Table 1 Home characteristics as univariate predictors of streptomycetes and endotoxin levels in household dust

		Geometric mean (geometric standard deviation)			
		Streptomycetes		Endotoxin	
Home characteristic	*n*	Concentration (cells/mg)	Load (cells/m^2)	Concentration (EU/mg)	Load (EU/m^2)
Direct factors/sources of bacterial contaminants					
Presence of cats[a]					
No	143	1531 (2.3)	6.94×10^5 (5.0)	148 (2.8)	6.70×10^4 (4.9)
Yes	35	2271 (2.1)	8.82×10^5 (3.3)	224 (3.2)	8.68×10^4 (5.3)
P		0.013	0.41	0.04	0.39
Presence of dogs[a]					
0	115	1533 (2.3)	6.18×10^5 (4.6)	158 (2.6)	6.39×10^4 (4.5)
1	46	1509 (2.3)	7.33×10^5 (4.2)	120 (2.6)	5.82×10^4 (4.6)
≥2	17	3557 (1.8)	2.16×10^6 (4.5)	381 (4.0)	2.32×10^5 (7.6)
P		<0.01	<0.01	<0.01	<0.01
Presence of plants[a]					
No	155	1604 (2.3)	7.41×10^5 (4.7)	157 (2.8)	7.27×10^4 (4.8)
Yes	23	2036 (2.3)	6.45×10^5 (4.3)	181 (3.5)	5.73×10^4 (5.9)
P		0.21	0.69	0.56	0.50
Number of inhabitants[b]					
≤ 3	25	1328 (2.5)	5.02×10^5 (7.3)	105 (2.1)	3.97×10^4 (5.1)
4	63	1817 (2.2)	6.51×10^5 (3.9)	169 (3.2)	6.07×10^4 (4.7)
≥5	90	1647 (2.3)	8.72×10^5 (4.5)	173 (2.8)	9.19×10^4 (4.9)
P		0.29	0.22	0.10	0.04
Indirect factors/sources of bacterial contaminants					
Relative humidity in PAR (%)[b]					
20–36	60	1979 (2.3)	1.09×10^6 (4.2)	130 (3.2)	7.12×10^4 (4.5)
37–46	62	1701 (2.3)	8.69×10^5 (4.3)	181 (2.4)	9.25×10^4 (4.2)
47–76	56	1325 (2.3)	3.89×10^5 (4.8)	176 (3.1)	5.17×10^4 (6.2)
P		0.04	<0.01	0.16	0.14
Temperature in PAR (/C)[b]					
18.9–22.5	59	2112 (2.3)	1.09×10^6 (4.4)	131 (2.9)	6.76×10^4 (4.6)
22.6–24.9	57	1624 (2.3)	8.31×10^5 (3.6)	168 (2.7)	8.62×10^4 (4.1)
25.0–30.6	62	1335 (2.3)	4.37×10^5 (5.4)	186 (3.0)	6.10×10^4 (6.2)
P		0.011	<0.01	0.16	0.49
Mold category[a]					
0	38	1749 (2.4)	7.99×10^5 (4.5)	171 (3.0)	7.80×10^4 (4.5)
1	121	1608 (2.2)	6.98×10^5 (4.5)	152 (2.9)	6.62×10^4 (5.2)
2	19	1775 (3.0)	7.86 × 105 (6.3)	195 (2.5)	8.62×10^4 (4.4)
P		0.81	0.87	0.59	0.73
ERMI[b]					
(−13.8)–(−0.34)	60	1430 (2.4)	6.77×10^5 (3.8)	149 (3.0)	7.06×10^4 (4.1)
(−0.35)–3.67	58	1578 (2.2)	5.89×10^5 (4.4)	138 (2.8)	5.14×10^4 (4.3)
3.68–22.69	60	2004 (2.4)	9.61×10^5 (5.8)	200 (2.8)	9.57×10^4 (6.3)
P		0.08	0.20	0.13	0.11
SLG2[b]					
8.13–16.85	59	1110 (2.4)	5.12×10^5 (4.2)	142 (3.1)	6.54×10^4 (4.8)
16.86–19.35	59	1769 (2.1)	6.91×10^5 (4.8)	159 (2.9)	6.22×10^4 (5.0)
19.36–27.05	60	2294 (2.1)	1.08×10^6 (4.6)	182 (2.6)	8.60×10^4 (5.1)
P		<0.01	0.03	0.43	0.49
Presence of mold smell[a]					
No	154	1651 (2.3)	7.30×10^5 (4.5)	152 (2.8)	6.70×10^4 (4.6)
Yes	24	1675 (2.5)	7.14×10^5 (5.8)	229 (3.4)	9.77×10^4 (7.5)
P		0.94	0.95	0.07	0.28
Season of dust sampling[a]					
Spring	27	1786 (2.6)	8.33×10^5 (4.1)	201 (4.8)	9.46×10^4 (5.0)
Summer	61	1198 (2.2)	3.64×10^5 (4.8)	192 (2.7)	5.83×10^4 (5.6)
Fall	61	1894 (2.2)	9.73×10^5 (4.5)	142 (2.5)	7.31×10^4 (5.1)
Winter	29	2289 (2.1	1.49×10^6 (3.1)	114 (2.2)	7.45×10^4 (3.5)
P		<0.01	<0.01	0.07	0.61
Central air conditioning[a]					
No	28	1492 (2.4)	6.42×10^5 (6.7)	211 (2.9)	9.08×10^4 (7.7)
Yes	150	1687 (2.3)	7.45×10^5 (4.3)	152 (2.9)	6.73×10^4 (4.5)
P		0.48	0.64	0.14	0.36

Table 1 (Continued)

		Geometric mean (geometric standard deviation)			
		Streptomycetes		Endotoxin	
Home characteristic	*n*	Concentration (cells/mg)	Load (cells/m^2)	Concentration (EU/mg)	Load (EU/m^2)
Use of dehumidifier[a]					
No	132	1624 (2.3)	7.99×10^5 (4.7)	157 (3.1)	7.75×10^4 (5.4)
Yes	45	1814 (2.3)	5.73×10^5 (4.4)	166 (2.5)	5.24×10^4 (3.7)
P		0.45	0.21	0.78	0.16
Humidifier use (wks/yr)[b]					
0	120	1743 (2.1)	7.92×10^5 (3.3)	178 (2.5)	8.08×10^4 (4.1)
1–20	27	1421 (2.5)	4.99×10^5 (4.3)	122 (2.5)	4.28×10^4 (3.7)
>20	31	1544 (2.4)	7.28×10^5 (5.1)	136 (3.0)	6.43×10^4 (5.4)
P		0.47	0.37	0.16	0.16
General home characteristics					
Year built[b]					
Before 1955	63	1839 (2.5)	6.43×10^5 (7.0)	206 (3.0)	7.20×10^4 (6.8)
1955–1985	57	1749 (2.2)	8.82×10^5 (3.5)	122 (2.6)	6.13×10^4 (4.1)
1986–	58	1428 (2.3)	6.85×10^5 (3.8)	164 (2.9)	7.85×10^4 (4.3)
P		0.22	0.51	0.03	0.69
Location of PAR[a]					
Bedroom	40	1238 (2.2)	5.45×10^5 (3.7)	129 (2.9)	7.51×10^4 (5.1)
Busy room	138	1800 (2.3)	7.91×10^5 (4.9)	171 (2.9)	5.67×10^4 (4.5)
P		0.014	0.18	0.14	0.33
Floor level of PAR[a]					
Basement	11	1914 (2.1)	1.08×10^6 (3.8)	127 (3.5)	7.17×10^4 (6.0)
1	129	1704 (2.4)	7.67×10^5 (4.8)	162 (2.8)	7.31×10^4 (5.0)
≥2	38	1437 (2.1)	5.43×10^5 (4.2)	165 (2.9)	6.23×10^4 (4.7)
P		0.47	0.32	0.74	0.86
Carpet in PAR[a]					
No	41	1654 (2.4)	3.86×10^5 (6.5)	180 (3.0)	4.20×10^4 (6.8)
Yes	137	1655 (2.3)	8.80×10^5 (3.9)	155 (2.8)	8.24×10^4 (4.3)
P		1.0	<0.01	0.43	0.017
Household income[a]					
<$40 000	51	1578 (2.5)	1.03×10^6 (5.6)	174 (3.1)	1.13×10^5 (5.6)
≥$40 000	125	1686 (2.3)	6.34×10^5 (4.2)	155 (2.8)	5.83×10^4 (4.5)
P		0.56	0.65	0.68	0.06

[a]Categorical variable.

[b]Linearly modeled variable. For this table categorized into approximate tertiles.

PAR, Child's primary activity room; ERMI, Environmental Relative Moldiness Index; SLG2, sum of log-transformed Group 2 mold concentrations.

only. Presence of cat and location of dust sampling was associated with streptomycetes concentration but not with load, whereas the presence of carpet in the sampling room was associated with streptomycetes load only.

As was the case for streptomycetes levels, the presence of two or more dogs was strongly associated with both endotoxin concentration and load. Homes with one or more cats present had higher endotoxin levels in both concentration and load than homes with no cats. Furthermore, the number of inhabitants in the household was significantly associated with both endotoxin concentration and load. By contrast with streptomycetes levels, neither season of dust collection nor SLG2 was a significant predictor of either endotoxin concentration or load. Temperature at the time of dust collection was correlated with endotoxin concentration but not with endotoxin load. Household income remained in the model for endotoxin load, with household incomes < $40 000 per year associated with elevated endotoxin loads. As for streptomycetes levels, the presence of carpet in the sampling room was associated with endotoxin load but not with concentration.

Discussion

The geometric mean of streptomycetes concentration in dust for all homes, 1655 cells/mg, was higher than means obtained in earlier studies of Finnish homes (Lignell et al., 2008; Rintala and Nevalainen, 2006). Differences in the sampling methods and in the processing of the dust may in part explain the higher levels measured in this study.

The geometric means for endotoxin concentration and load, 160 EU/mg and 7.1×10^4 EU/m^2, respectively, were higher than those found in other studies of US homes. In a nationwide study, Thorne et al. (2009) obtained a geometric mean of 63.9 EU/mg for endotoxin concentration and 2×10^4 EU/m^2 for endotoxin

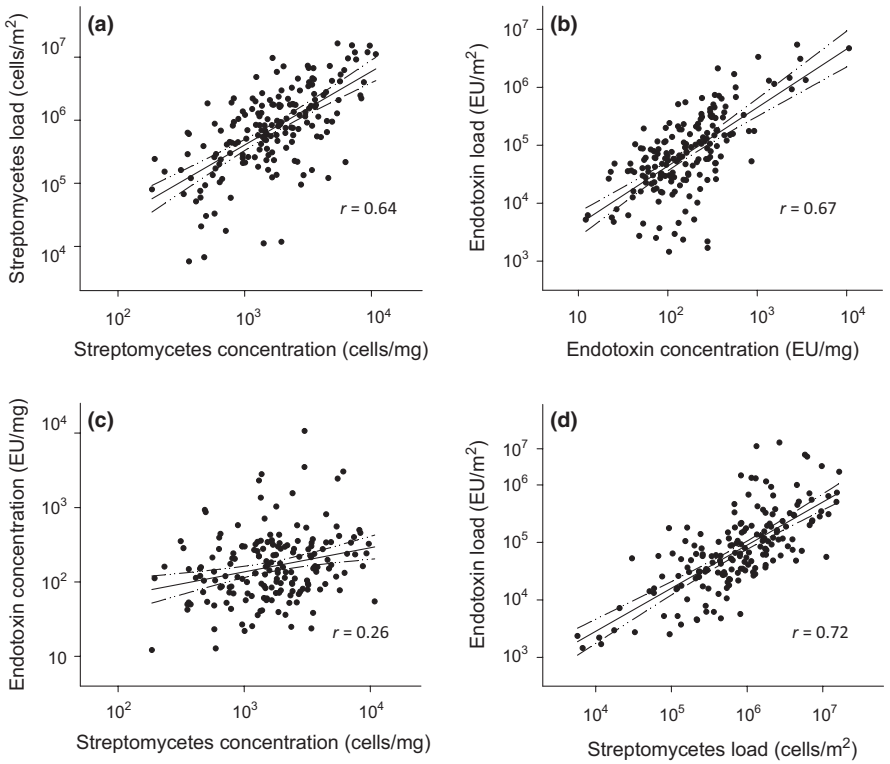

Fig. 1 Correlations between exposure outcome variables. Dotted lines are 95% confidence lines. Correlation coefficients are based on Spearman product moment correlations between log-transformed outcome variables. (a) Correlation between streptomycetes concentration and streptomycetes load. (b) Correlation between endotoxin concentration and endotoxin load. (c) Correlation between streptomycetes concentration and endotoxin concentration. (d) Correlation between streptomycetes load and endotoxin load

load on family room floors. Similarly, Abraham et al. (2005) obtained a geometric mean of 83 EU/mg for endotoxin concentration on family room floors in a study of 470 households in Boston, Massachusetts. Poor interlaboratory reproducibility of the *Limulus* assay of endotoxin in dust has been demonstrated previously (Milton et al., 1997). In addition, different dust sampling methodologies may have played a part.

Concentrations were significantly correlated with loads for both streptomycetes and endotoxin. Corresponding correlation coefficients were 0.64 and 0.67 indicating that less than half of the variation in concentration could be explained by load and *vice versa*. Contaminant levels are most often expressed as concentrations, amount of contaminant per dust weight unit. Load, which is the amount of contaminant per area unit, may be a more useful measure of contaminant levels because it represents the total burden of the contaminant in the home. Homes with the same load may have very different concentrations, depending on the amounts of different types of particulate matter in the dust. The correlation between streptomycetes load and endotoxin load was significant with $r = 0.72$ ($P << 0.01$), meaning that approximately half of the variability of streptomycetes load could be explained by endotoxin load and *vice versa*. This suggests that streptomycetes and endotoxin in part have the same sources. The correlation between streptomycetes concentration and endotoxin concentration was much weaker than the correlation between loads. Previous studies have similarly found that levels of different microbial contaminants expressed as loads are more highly correlated than the same levels expressed as concentrations (Gehring et al., 2001; Iossifova et al., 2008). The reason for this discrepancy is not clear but may be explained by the large variation in the amount of total fine dust collected from the floor.

Both streptomycetes concentration and load were significantly lower in dust collected in the summer compared to other seasons, and season of sampling remained in the multivariate analyses as a strong predictor of streptomycetes levels in dust. The home visits for this study occurred over a time period of 2 years, and when each year was analyzed separately, streptomycetes levels were significantly lower in dust collected in the summer for both years (data not shown). This suggests that our results represent a real seasonal pattern. A denser cover of vegetation in the summer may play a role by covering soil on the ground and thereby limiting transport of soil indoors.

High temperature and relative humidity in PAR at the time of sampling were, somewhat counterintuitively, associated with decreased levels of streptomycetes

Table 2 Multiple regression models for predicting streptomycetes and endotoxin levels in household dust as functions of home characteristics

	Adjusted means ratio[a] (95% CI)			
	Streptomycetes		Endotoxin	
Home characteristic	Concentration	Load	Concentration	Load
Summer	1	1		
Fall + spring	1.50 (1.18, 1.92)	2.57 (1.58, 4.20)		
Winter	1.86 (1.37, 2.53)	3.90 (2.33, 6.52)		
Presence of cats				
No	1		1	1
Yes	1.42 (1.09, 1.85)		1.45 (1.03, 2.06)	1.77 (1.02, 3.06)
Presence of dogs				
<2	1	1	1	1
≥2	2.32 (1.58, 3.41)	2.79 (1.34, 5.83)	2.45 (1.50, 4.02)	4.65 (2.11, 10.22)
Number of inhabitants (+1)			1.34 (1.15, 1.57)	1.64 (1.26, 2.13)
Temperature (+5°C)			1.32 (1.13, 1.53)	
ERMI (+1 unit)	1.01 (1.00, 1.03)	1.03 (1.00, 1.06)	1.02 (1.00, 1.04)	
SLG2 (+1 unit)	1.08 (1.05, 1.12)	1.1 (1.04, 1.17)		
Location of PAR				
Bedroom	1			
Busy room	1.38 (1.07, 1.78)			
Carpet in PAR				
No		1		1
Yes		2.66 (1.66, 4.28)		1.84 (1.11, 3.04)
Household income				
<$40 000			1	1
≥$40 000			0.78 (0.58, 1.05)	0.38 (0.23, 0.63)

[a]Ratios of antilogs of predicted means were calculated, which were adjusted for other variables in the model. Predicted means were based on parameter estimates from the multiple regression analysis.

PAR, Child's primary activity room; ERMI, Environmental Relative Moldiness Index; SLG2, sum of log-transformed Group 2 mold concentrations.

concentration and load. These two variables did not remain in the multivariate models, likely because of their association with season.

Several large studies have failed to detect an association between endotoxin levels and season (Bischof et al., 2002; Thorne et al., 2009), although Thorne et al. point out that the lack of an association in their study could be a result of the variety of different climate types in the United States. Abraham et al. (2005) in their study of homes in the Boston, Massachusetts, area reported significantly lower concentrations of dust endotoxin in the fall and winter relative to summer. In agreement with the Boston study, we found a trend toward lower endotoxin concentrations in the fall and winter in the univariate analyses.

Neither endotoxin nor streptomycetes was associated with the categories for visible mold. In contrast, streptomycetes concentration and load, as well as endotoxin concentration, showed trends toward associations with ERMI. Streptomycetes concentration and load, however, were more strongly and significantly associated with SLG2, which is a measure of the levels of ten indicator mold species not associated with water damage and believed to have outdoor sources (Vesper, 2010). This is not surprising, considering that most *Streptomyces* species are ubiquitous soil dwellers (Kutzner, 1986). Although certain *Streptomyces* species have frequently been isolated from water-damaged building materials (Rintala et al., 2002; Suihko et al., 2009), our results suggest that the *Streptomyces* genus as a whole is too diverse to be a useful indicator of water damage in Midwestern US homes. The same may be true for Gram-negative bacteria as represented by endotoxin. More specific assays of individual bacterial species may be more informative.

A large number of earlier studies have found that the presence of dogs is one of the strongest predictors of elevated endotoxin levels in household dust, and the presence of dogs has also been shown to be associated with endotoxin levels in indoor air (Bischof et al., 2002; Park et al., 2001; Thorne et al., 2009). We showed that, similar to endotoxin, the strong association with the presence of dogs held for streptomycetes concentration and load as well. Intriguingly, for both endotoxin and streptomycetes, the association was for two or more dogs, whereas one dog made no difference. Similar associations with two or more dogs have been found earlier for levels of the mold *Alternaria* in indoor dust (Cho et al., 2006), and a study of endotoxin levels and the presence of dogs as predictors of wheezing and atopy in infants showed that high endotoxin exposure was associated with reduced wheezing, but only in the presence of two or more dogs (Campo et al., 2006). It is possible that having two or more dogs may be associated with family habits distinct from those of families with one or no dog. Another explanation

might be that two or more dogs roughhouse more and thereby shed more microbial contaminants adhering to fur and skin cells. The presence of cats remained in the models as a predictor for all outcomes except streptomycetes load, and these results agree with most previous studies (Bischof et al., 2002; Gereda et al., 2001; Thorne et al., 2009).

Number of inhabitants was a predictor of both endotoxin concentration and load in dust, and these results agree with the findings reported by Thorne et al. (2008). Other studies have not detected a significant association between endotoxin levels and number of inhabitants (Bischof et al., 2002; Gereda et al., 2001). Given that transport of streptomycetes from outside soil is likely to play a role in determining indoor levels, it may have been expected that streptomycetes levels as well would be associated with number of inhabitants, but that was not the case. On the other hand, transport of streptomycetes from outside may explain why streptomycetes concentration was significantly associated with the location of the PAR, with busy rooms (living rooms or family rooms) having higher concentrations than bedrooms. Bedrooms are usually located further away from the home entrance.

The presence of carpet in PAR was a strong predictor of both streptomycetes and endotoxin loads, but not concentrations, and this is easily explained by the fact that carpet traps dust and dirt to a much higher degree than smooth floors do. To collect enough dust from smooth floors usually it was necessary to vacuum an entire room. Therefore, at a given concentration of contaminant in dust, carpeted floors will usually contain higher total amounts of contaminant per area unit.

Income remained in the multivariate models as a predictor of endotoxin levels; both concentration and load were higher in homes with lower family income, although for concentration, the association was not statistically significant. This is in line with previous studies and has been explained by the associations between income and poor housing conditions (Thorne et al., 2009). Interestingly, streptomycetes *levels* were not associated with income. If streptomycetes found indoors mostly have outdoor sources, then streptomycetes levels can be expected to be associated with activities and factors such as gardening, spending time outdoors, and children and pets running in and out, which are not necessarily associated with income. In this study, there was no significant association between income and the presence of dogs.

The limitations of this study include the use of floor dust as a proxy measure of air exposure. The advantage of this approach is that the collected dust sample can be assumed to be reflective of a longer time period than samples obtained using air samplers, and dust samples usually have far less temporal variability. The validity of dust sampling as a proxy measure of airborne microbial contaminants has been demonstrated in studies showing associations between respiratory health outcomes and levels of dust borne fungi and/or endotoxin (Park et al., 2006; Vesper et al., 2007). Nevertheless, microbial contaminants in dust can also be expected to be brought indoors from outdoor sources through ground trafficking by people and pets, and the extent to which those contaminants become aerosolized is not well understood.

The study was geographically limited to the greater Cincinnati area of the Ohio valley, and the results may not be applicable to other regions of the country. Indeed, a recent study (Thorne et al., 2009) showed that geographic location is a strong predictor of both endotoxin concentration and endotoxin load in household dust.

Conclusions

Streptomycetes levels in house dust were significantly associated with levels of ubiquitous outdoor molds, whereas only a borderline association with the ERMI and no association with the presence of visible indoor mold were found. This finding suggests that although individual *Streptomyces* species have frequently been isolated from moisture-damaged building material, the *Streptomyces* genus as a whole may be too diverse to serve as an indicator of moisture damage. Season of dust collection and the levels of outdoors molds were strong predictors of streptomycetes levels, which may indicate that indoor streptomycetes mostly have outdoor sources. In contrast to streptomycetes, endotoxin was not associated with the season of dust collection or the levels of outdoor molds, but was associated with the number of inhabitants and household income. A partial explanation of the results may be that streptomycetes is dominated by outdoor sources, whereas indoor sources are more important for endotoxin. Future research is needed to explore possible health implications of variations in indoor streptomycetes levels.

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