Dustborne and Airborne Gram-Positive and Gram-Negative Bacteria in High versus Low ERMI Homes

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Abstract

The study aimed at investigating Gram-positive and Gram-negative bacteria in moldy and non-moldy homes, as defined by the home's Environmental Relative Moldiness Index (ERMI) value. The ERMI values were determined from floor dust samples in 2010 and 2011 and homes were classified into low (<5) and high (>5) ERMI groups based on the average ERMI values as well as 2011 ERMI values. Dust and air samples were collected from the homes in 2011 and all samples were analyzed for Gram-positive and Gram-negative bacteria using QPCR assays, endotoxin by the LAL assay, and N-acetyl-muramic acid using HPLC. In addition, air samples were analyzed for culturable bacteria. When average ERMI values were considered, the concentration and load of Gram-positive bacteria determined with QPCR in house dust, but not air, were significantly greater in high ERMI homes than in low ERMI homes. Furthermore, the concentration of

endotoxin, but not muramic acid, in the dust was significantly greater in high ERMI than in low ERMI homes. In contrast, when ERMI values of 2011 were considered, Gram-negative bacteria determined with QPCR in air, endotoxin in air, and muramic acid in dust were significantly greater in high ERMI homes. The results suggest that both short-term and long-term mold contamination in homes could be linked with the bacterial concentrations in house dust, however, only the current mold status was associated with bacterial concentrations in air. Although correlations were found between endotoxin and Gram-negative bacteria as well as between muramic acid and Gram-positive bacteria in the entire data set, diverging associations were observed between the different measures of bacteria and the home moldiness. It is likely that concentrations of cells obtained by QPCR and concentrations of cell wall components are not equivalent and represent too broad categories to understand the bacterial composition and sources of the home microbiota.

Keywords

Indoor air quality; Bacteria; Mold; ERMI; Endotoxin; Muramic acid

INTRODUCTION

The association between bacterial contamination and respiratory health has lagged behind studies of mold contamination and health (WHO, 2009). Human exposures to bacteria and their components in water-damaged homes have been reported to result in atopic and non-atopic inflammatory diseases (Douwes et al., 2003). Quantifying bacterial populations and their components in non-water-damaged and water-damaged moldy homes is needed to better understand building-related illnesses. Gram-positive and Gram-negative bacteria, including such genera as *Acinetobacter, Bacillus*, *Flavobacterium*, *Nocardia*, *Thermomonospora* and *Streptomyces*, have been isolated from moisture-damaged building materials and dust (Suihko et al., 2009; Rintala et al., 2008; Torvinen et al., 2006; Peltola et al., 2001; Andersson et al., 1997).

The major cell wall component of Gram-negative bacteria is lipopolysaccharide/endotoxin (hereafter, endotoxin), whereas N-acetyl-muramic acid (hereafter, muramic acid) is the major cell wall component of Gram-positive bacteria. For pathogenic bacteria, these components are important virulence factors (Feezor et al., 2003) but can also cause inflammatory responses that have been associated with both increased and decreased risk of the development or exacerbation of allergy and asthma (Heederik and von Mutius, 2012). Therefore, in addition to mold exposures, bacterial growth may affect occupant health in water-damaged homes but the quantification of bacteria has not been standardized as it has been for molds.

The quantification of mold contamination was standardized by using a DNA-based analytical method and the Environmental Relative Moldiness Index (ERMI) scale (Vesper et al., 2011). Developed by United States Environmental Protection Agency (US EPA) researchers in collaboration with the Department of Housing and Urban Development (HUD), the ERMI scale relies on measuring the concentrations of 26 mold species

indicating water-damage (Group 1) and 10 species that were found in randomly selected non-water-damaged homes during the nationwide HUD 2006 American Healthy Homes Survey (Group 2) (Vesper et al., 2007). The ERMI scale was divided into quartiles and an ERMI value greater than 5 is in the upper quartile, indicating the highest mold contamination for the U.S. homes. Exposure of infants to high ERMI homes has been associated with the development of asthma (Reponen et al., 2012; 2011).

In this study, we investigated Gram-positive and Gram-negative bacteria, as well as their cell wall constituents, muramic acid and endotoxin, in dust and air samples collected from low and high ERMI homes. Both groups of bacteria were quantified using quantitative PCR (QPCR) and traditional cultivation methods. We also determined correlations between the measures of bacteria and some of the home's environmental conditions, including temperature, relative humidity (RH), age of the home, and the number of occupants.

MATERIALS AND METHODS

Study Homes

The families recruited for this study (n=42) were a subset of the cohort participating in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) undertaken in Cincinnati, Ohio and Northern Kentucky in 2001 (Ryan et al., 2007; LeMasters et al., 2006). The ERMI values for these homes had been determined in 2010 (Reponen et al., 2012; 2011). Based on the ERMI scale, homes were selected for inclusion into a low ERMI (<5) group (n=21) or a high ERMI (>5) group (n=21). With the possibility that conditions had changed, dust was again collected in 2011 from the same homes and the ERMI analysis was repeated [all primer and probe sequences used in the ERMI QPCR assays are available online (US Environmental Protection Agency, 2012)]. Subsequently, the homes were reclassified into low (n=25) and high (n=17) ERMI groups based on the average ERMI for the two years and into low (n=31) and high (n=11) ERMI groups based solely on year 2011 ERMI values. The protocols for collection and analysis of household dust samples previously approved by the Institutional Review Board at the University of Cincinnati were followed during the home sampling.

On-site Home Visit and Sampling

On-site home visits were performed by two-person teams. Floor dust samples were obtained for the assessment of bacteria and ERMI in the child's primary activity room (PAR), as described by Cho et al. (2006). Dust samples were collected with a vacuum cleaner (Filter Queen Majestic; HMI Industries Inc., Seven Hills, OH) at a flow rate of 800 l/min. A custom-made cone-shape HEPA filter trap (Midwest Filtration, Cincinnati, OH) with a collection efficiency exceeding 95% for particles larger than 0.3 μm was attached to the nozzle of the vacuum cleaner to collect the dust sample. For carpeted floor, dust samples were collected from an area of 2 m^2 in the middle of the room at a vacuuming rate of 2 min/m^2. For non-carpeted floor (hard wood, linoleum, tile, or plastic sheet floor), the entire room floor was vacuumed at a rate of 1 min/m^2. Large dust particles were removed by sieving (355 μm mesh sieve), and the resulting dust was stored at −20°C before analyses. Temperature (°C) and RH (%) were recorded in each home by a portable thermo-hygrometer

(Fisher Scientific, Pittsburgh, PA) and data on the number of occupants and age of the homes were collected through a questionnaire survey.

Air samples (5.47 ± 0.54 m^3 air) were collected at 3.5 l/min over a 24-h period using a NIOSH-developed 2-stage cyclone sampler, which separates airborne particles into three size fractions: < 1.0 μm, 1.0–1.8 μm, and >1.8 μm (Lindsley et al., 2006). Dust and air samples were analyzed for muramic acid and endotoxin biochemically and for Gram-positive and Gram-negative bacteria using QPCR. Four air samples had insufficient amounts of extract available for QPCR assays.

DNA Extraction from Environmental Samples

An aliquot of 5.0 ± 0.1 mg of each dust sample was extracted by placing the sample in a 'bead-beating' tube with glass beads (Sigma glass beads G-1277; size: 212 – 300 μm; 0.3 g in each tube) and shaken for 1 min, as previously described (Haugland et al., 2004; 2002). The DNA was purified using the DNA-EZ extraction kit (GeneRite, Cherry Hill, NJ). The air samples were extracted as described in Singh et al. (2011). Each size fraction was separately analyzed and the results were combined to represent non-size-selective air concentrations for bacterial and bacterial cell components.

QPCR Analysis of Gram-Positive and Gram-Negative Bacterial Cells

QPCR assays of Gram-positive and Gram-negative bacteria were conducted separately. The primer and probe sequences for groups of bacteria are presented in Table 1. These groups were previously analyzed collectively by Kärkkäinen et al. (2010) using the same primers and probes; the amplicon sizes are also available in the quoted paper. Our attempt to perform the original combined assay was unsuccessful, perhaps due to the different sequence detector utilized. The QPCR assays targeted the 16s rRNA gene. Primers and probes were synthesized commercially (Applied Biosystems, Inc., Foster City, CA) and final concentrations were 1 μM for each primer and 80 nM for each probe.

Each QPCR reaction contained 12.5 μl of "Universal Master Mix" (Applied Biosystems, Inc.), 1 μl of a mixture of forward and reverse primers at 25 μM each, 2.5 μl of a 400 nM TaqMan probe (Applied Biosystems, Inc.), 2.5 μl of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO), 1.5 μl of DNA free water (Cepheid, Sunnyvale, CA), and 5 μl of DNA extract from the sample (DNA from 5.0 ± 0.1 mg of dust extracted in a final volume of 200 μl and then 5 μl of that was used here for analysis). Assays were performed using the Roche LightCycler® 480 System (Roche Applied Science, Indianapolis, IN) following manufacturer's instructions.

The QPCR program for the Gram-positive assay consisted of an initial incubation step at 95°C for 15 min, followed by 45 cycles of denaturation at 95°C for 15 s and annealing/extension at 57°C for 45 s. The QPCR program for the Gram-negative assay 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. Forty-five cycles were chosen because this was the Roche instrument standard protocol for use of the 2nd derivative in calculating the cycle threshold.

Standard curves were generated from pure cultures of *Bacillus subtilis* (ATCC 6051, American Type Culture Collection, Manassas, VA) and *Escherichia coli* (ATCC 25922) for the Gram-positive and Gram-negative assays, respectively. Cell numbers were based on hemacytometer (Hausser Scientific, Horsham, PA) counts in the highest concentration in the standard curve. DNA extracted from the highest concentration of cells in the standard curve was used to generate a dilution series for the standard curve. Positive controls (extracts of *B. subtilis* or *E. coli* cells) and negative controls (Cepheid DNA free water) were run with each assay mix. The internal control was the addition of a known concentration of *Geotrichum candidum* cells to each sample before extraction (Haugland et al., 2004). The analysis was discarded and repeated if any abnormalities observed. Detection limits per PCR reaction, defined at a C_t value of 40, were 82 cells for *B. subtilis* and approximately one cell for *E. coli*.

Bacterial dust concentrations were expressed as average cell equivalents per milligram dust. Bacterial dust loading, expressed as cell equivalents per m^2 floor area, was derived from concentration by multiplying concentration with total mass of dust vacuumed and dividing by m^2 floor area vacuumed. Bacterial air concentrations were expressed as cell equivalents per m^3 of air sampled.

Analysis of Endotoxin and Muramic Acid

Dust and air samples were analyzed for endotoxin using the *Limulus* Amebocyte Lysate assay (LAL; Pyrochrome LAL; Associates of Cape Cod Inc, Falmouth, MA), as described previously (Adhikari et al., 2010; 2009). An aliquot of 25 mg of sieved dust was used for each analysis. The samples were spiked with endotoxin standard of 0.50 EU/ml to assure that there was no inhibition or enhancement between the extract and the reagents. Endotoxin concentrations were expressed as endotoxin units per mg of dust (EU/mg). The lower detection limit (LDL) for endotoxin was 0.002 EU/mg in dust and 0.076 EU/m^3 in air. The concentrations in all measured dust samples were above the LDL.

For analyzing muramic acid, vacuum dried dust (100 mg) and air sample extracts (1.0 ml) were hydrolyzed with 6 N HCl at 95°C for four hours. After cooling, centrifuging, and drying-off the HCl under vacuum, the amino acid/amino sugar analysis was performed by neutralization with triethylamine, modification of free amino groups with phenyl isothiocyanate (PITC), and high-performance liquid chromatography (HPLC) separation with detection by absorbance at 254 nm. The detection limits were 0.1 ng/mg for dust and 0.1 ng/m^3 for airborne muramic acid.

Culturing Bacteria from Air Samples

For comparison to QPCR analyses, concentrations of culturable airborne Gram-positive and Gram-negative bacteria were estimated by collecting air samples using two-stage Andersen samplers (Thermo Andersen, Franklin, MA) equipped with Trypticase soy agar (for all bacteria) and MacConkey agar (for Gram-negative bacteria) plates. The counts of Gram-positive bacterial colonies were estimated by subtracting the number of Gram-negative colonies from total number of colonies. The samples were collected at an air flow rate of 28.3 ± 2 l/min for 15 min. The agar plates were subsequently incubated at two incubation

temperatures: at $30 \pm 2°C$ for a minimum of three days for mesophilic bacterial species or at $55 \pm 2°C$ for a minimum of seven days for thermophilic bacterial species (US EPA, 2003). The colonies were counted by a using a Quebec darkfield colony counter (Cambridge Instruments, Inc., Buffalo, NY). The counts of mesophilic and thermophilic colonies were combined to obtain the final concentration in colony forming units. Concentrations of culturable bacteria in air samples were described as colony forming units per m^3 air (CFU/m^3).

Statistical analyses

Independent samples *t*-test was conducted to examine the differences in means between two ERMI-specific groups for different bacterial variables after log transformation. The non-parametric Spearman's correlation coefficients were calculated between different bacterial and environmental variables. Non-parametric method was employed because the data were not normally or log-normally distributed in several cases when data for ERMI-specific and all homes were considered. Statistical analyses were performed using SPSS Statistics 17.0 (IBM Corporation, Armonk, NY) and SAS 9.3 software (SAS Institute, Carry, NC).

RESULTS

According to 2011 ERMI assessment, 31 homes belonged to the low ERMI group and 11 homes belonged to the high ERMI group. The average ERMI value over two years (2010 and 2011) was determined for each home to provide a longer term view of the conditions in the home and resulted in 25 low ERMI and 17 high ERMI homes.

The differences between low and high ERMI homes in the Gram-positive and Gram-negative bacterial concentrations for dust and air samples are shown in Table 2a (based on average ERMI) and Table 2b (based on 2011 ERMI values). When average ERMI values were considered, both the concentration and load of Gram-positive bacteria in the dust were significantly greater (*t*-test p <0.001 and 0.003, respectively) in high ERMI homes than in low ERMI homes (Table 2a). The same trend was observed for the concentration and load of Gram-negative bacteria in dust, but the differences were not statistically significant (Table 2a). Furthermore, none of the air concentrations were significantly different in high versus low ERMI homes separated according to the average ERMI values.

The results were somewhat divergent when high ERMI and low ERMI groups were separated based on 2011 ERMI values (Table 2b). Although increasing trends of Gram-positive and Gram-negative bacterial concentrations were observed in dust samples of high ERMI homes, the differences were not statistically significant. Only total cell concentrations of Gram-negative bacteria in air were significantly higher in high ERMI homes than low ERMI homes.

The total cell concentrations in the air as determined by QPCR were on average about 2,000 (SD=9,600) times higher than the concentrations of culturable bacteria. Furthermore, concentrations of Gram-positive bacteria were higher than Gram-negative bacteria in both high and low ERMI homes in dust as well as in air samples (Tables 2a and 2b). The ratios

between average concentration of Gram-positive and Gram-negative cell concentrations in all homes were 2.9 in dust samples and 35.8 in air samples.

Tables 3a and 3b show the differences in the measures of the cell wall components of Gram-positive bacteria (muramic acid) and Gram-negative bacteria (endotoxin) between low versus high ERMI homes, when average ERMI and 2011 ERMI values were considered, respectively. When the judgment was made solely based on average ERMI values, the only significant difference found between the high ERMI and low ERMI groups of homes was for the concentration of dust endotoxin, which was greater in the high ERMI homes. However, when 2011 ERMI values were considered, endotoxin concentration in air and muramic acid concentration in dust were significantly higher in high ERMI homes.

When assessing correlations between the various bacterial measures, the data were not separated into high and low ERMI groups because these measures are related to bacteria itself and not likely to be dependent on ERMI categories. The dust concentrations of both Gram-negative and Gram-positive bacteria significantly correlated with dust loading for all homes (Table 4). However, neither the dust concentration nor the dust loading correlated with the respective air concentration.

The correlations between bacterial cell wall components and concentration of bacterial cells in dust and air samples for all homes are presented in Table 5. Distributions of data in selected correlations are presented in Figure 1. The dust loading and air concentration of Gram-negative bacteria significantly correlated with respective measure of endotoxin in all homes (Table 5A, Figure 1a, 1b). In addition, there was a significant correlation between Gram-positive cell concentration and muramic acid concentration in dust (Table 5B, Figure 1c) and between Gram-positive cell loading and muramic acid loading (Table 5B, Figure 1d). We also examined the correlations between levels of culturable Gram-positive and Gram-negative bacteria with total Gram-positive and Gram-negative cells measured by QPCR as well as levels of muramic acid and endotoxin (dust concentration, loading, and air). Only muramic acid in air significantly correlated with culturable Gram-positive bacteria in air ($\rho = 0.002$; $p < 0.001$, data not shown).

The correlations between measures of Gram-negative and Gram-positive bacterial concentrations and some potentially relevant environmental variables in all homes are shown in Table 6. Among the Gram-negative bacterial measures in dust, positive correlations were found between cell concentrations and RH (range: 21 to 57%, average: 36%) (Figure 1f) as well as between endotoxin and age of home (7 to 128 years, average 57 years). Culturable Gram-negative bacteria correlated positively with both RH and temperature (range: 18.6 to 27.4°C, average: 23.3°C). None of the Gram-negative bacterial measures correlated with the number of occupants. Also, none of the bacterial measures of dust loading correlate with environmental variables, except for a significant inverse correlation ($\rho = -0.325$; $p = 0.035$) between the loading of Gram-positive bacteria with temperature (data not shown).

For Gram-positive bacterial measures, concentrations of total Gram-positive bacteria and muramic acid in dust inversely correlated with temperature in the homes. In contrast,

culturable airborne bacteria in air positively correlated with temperature and RH. The concentration of Gram-positive bacteria in the air samples positively correlated with the number of occupants in the homes (range: 2 to 9, average: 5) (Figure 1e) but muramic acid concentration in dust inversely correlated with the occupants. Age of home did not seem to be significant factors in controlling the Gram-positive bacterial concentrations (Table 6).

DISCUSSION

The results suggest that both short-term and long-term mold contamination in homes could be linked with the bacterial concentrations in house dust; however, only the current mold status was associated with bacterial concentrations in air. This was likely due to different levels of environmental tolerance among molds and bacteria when they are growing in aerosolizable dust in moisture damaged home environments. Diverging associations were observed between the home moldiness and different measures of bacteria. Long-term mold contamination was associated with Gram-positive bacteria when measured with QPCR, but not when measured as a cell wall component (muramic acid), and with Gram-negative bacteria when measured as cell-wall component (endotoxin), but not when measured by QPCR. This contradictory observation indicates that cell concentrations obtained by QPCR and concentrations of cell wall components may not represent the same bacterial identity and represent too broad categories to understand the bacterial composition and sources of the home microbiota. Another possible explanation is that molds and bacteria growing in water damaged building materials could have different levels of tolerance for environmental stresses. Short-term water damage in building materials can support both mold and bacteria, but with time, the level of bacterial contamination may change faster than the mold contamination. Gram-positive bacteria can grow in parallel with molds because they are more tolerant to dry conditions (Møretrø et al., 2010; Janning and in't Veld, 1994) than Gram-negative bacteria, whose growth could be diminished when building material is drying. This explanation seems feasible because we found that current moldiness was associated with airborne Gram-negative bacterial concentration.

Although the dust samples from high ERMI homes contained significantly greater concentrations of endotoxin, we did not find a significant difference in the concentration of Gram-negative bacteria in the dust samples between high and low ERMI homes. Similarly, dust concentrations of endotoxin and gram-negative bacteria did not correlate with each other. One reason for this finding may be that different species of Gram-negative bacteria produce different amounts of endotoxin (Weber-Frick and Schmidt-Lorenz, 1988). The amount of endotoxin might depend on the species present in low versus high ERMI homes and some Gram-negative species could be present in higher concentration in moldy homes. For example, Kettleson et al. (2013) found that the Gram-negative bacterium, *Stenotrophomonas maltophilia*, occurred in significantly higher concentrations in high ERMI homes versus low ERMI homes. Interestingly, in air samples, endotoxin and Gram-negative bacteria correlated and both were also higher in high ERMI homes. These observations support the previous studies indicating that exposure assessment based on dust versus air samples could provide different perspectives (Adhikari et al., 2010). Another observation of our study was that the age of a home was significantly correlated with the endotoxin concentration in dust but not with the other bacterial measurements. This

observation is consistent with the previous reports on endotoxin levels in homes, for example, a large study in German homes demonstrated that endotoxin concentrations were higher in old buildings (means ratio = 1.52, 95% C.I.: 1.14; 2.04; Bischof et al., 2002). Older homes may favor Gram-negative bacteria (Kettleson et al., 2013) due to accumulation of dust serving as growth substrate. Endotoxin can remain in homes for longer period because it is a stable molecule.

Good correlations were found in both dust concentration and dust loading between muramic acid and Gram-positive bacteria. Therefore, somewhat surprisingly, differing associations were found between Gram-positive bacterial measures and occupancy. As expected, occupancy was positively correlated with Gram-positive bacteria in air, possibly due to contributions of Gram-positive bacteria from skin, but negatively with muramic acid in dust because Gram-positive bacterial community could be largely different in dust. Furthermore, differences in growth rate and developmental state of the Gram-positive bacteria between human body and dust can also affect cell wall composition of Gram-positive bacteria (Chien et al., 2012) including muramic acid.

Home temperature was inversely correlated with the dust concentrations of Gram-positive bacteria and muramic acid. How typical temperature range in home environments affect Gram-positive bacterial growth in house dust is quite unknown. Our observations suggest that home temperature may have a negative influence on muramic acid content of Gram-positive bacteria in dust. On the other hand, when culturable bacteria were considered, both Gram-positive and Gram-negative bacteria in air were positively correlated with home temperature, which is consistent with observations reported earlier (Obbard et al., 2000).

Home RH positively influenced Gram-negative, but not Gram-positive bacteria, analyzed by QPCR. However, for airborne culturable bacterial concentrations, both Gram-positive and Gram-negative bacteria demonstrated positive correlations with RH, similar to previous report (Aydogdu et al., 2005). Although both Gram-negative and Gram-positive bacteria have been shown to persist under desiccating conditions, previous studies suggest that Gram-positive bacteria exhibit enhanced tolerance to dry conditions compared to Gram-negative bacteria (Møretrø et al., 2010; Janning and in't Veld, 1994). This may explain why we found a greater portion of Gram-negative bacteria in dust than in air samples. However, all of these observations should be confirmed in larger studies because of some acknowledged limitations of the present study.

One limitation of the study is associated with changing of the ERMI status for 17 homes over the year. The participating families in this study were mainly low-income, living in older, often rental, properties (Reponen et al 2013). If water problems develop in the home, the family may not have resources for or control over timely repairs. In 13 homes, the ERMI values had changed to low ERMI status in 2011 indicating that an intervention, e.g. repairs or carpet replacement may have occurred. However, in four homes, the ERMI values were found to have changed into high ERMI status by the second sampling in 2011, suggesting increased home moldiness. The long-term associations between various bacterial contaminants might be clearer if the families were more stable and lived in well maintained homes. Another limitation of our study is that the quantification of Gram-positive and

Gram-negative bacteria was conducted using only *B. subtilis* and *E. coli* as standards, respectively. This is a common problem with studies that try to quantify by QPCR large and diverse groups of microorganisms. This investigation was also limited to only a few environmental factors that might affect bacterial populations and statistical adjustment with confounding factors was unattainable due to the limited number of homes sampled.

Although the dust-based ERMI values in infant's homes have been found to be predictive of the development of asthma (Reponen et al., 2011), it is unclear what dust bacterial concentrations mean in terms of bacterial exposure and health outcomes. However, cell products may be important in understanding these outcomes. Further long-term studies on ecology of mold and bacteria with increased number of samples are required to obtain more clarification. For example, enrichment of different bacterial species in dust and air samples and relationship between diversity of molds and bacteria could be investigated by applying high-throughput DNA sequencing methods.

In conclusion, multiple measures of bacterial populations may be needed to evaluate their impact on human exposures. It may also be useful to target specific species of bacteria for assessment rather than large categories of bacteria, like Gram staining status.

Acknowledgments

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Highlights

- High ERMI and low ERMI homes differ in bacterial contaminant levels.

- Only the current mold status is associated with bacterial concentrations in air.

- Dusts versus air samples provide different perspectives of bacterial contamination.

- Occupancy in homes correlates with total Gram-positive bacterial cells in air.

- Correlations of temperature and RH with culturable and total bacteria are unlike.
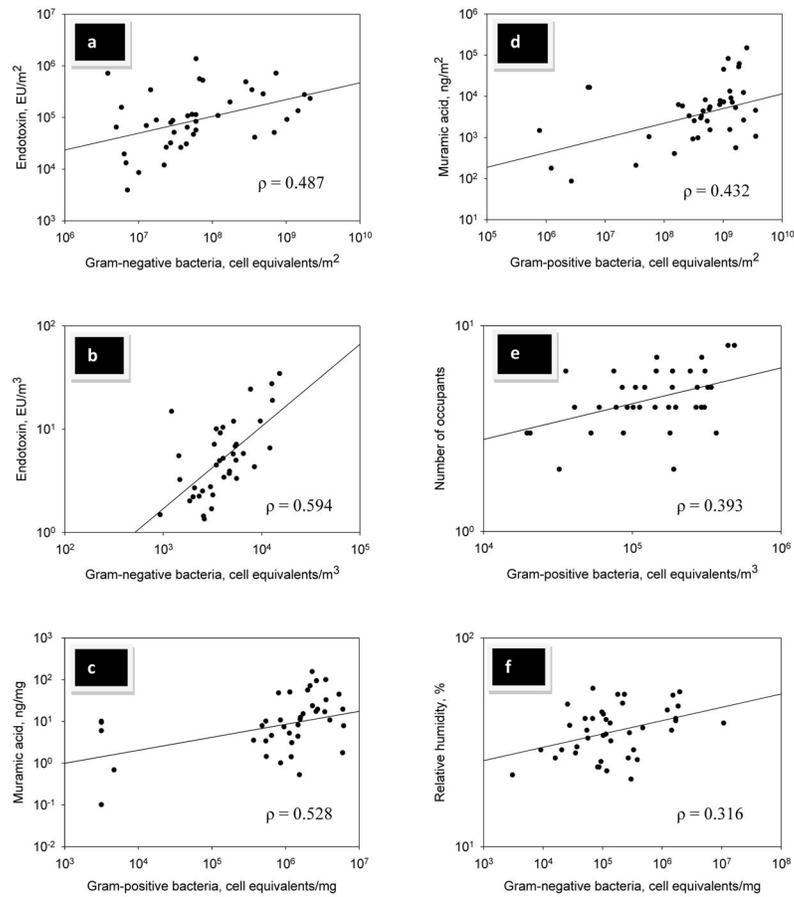
Figure 1.
Selected significant (p<0.05) correlations observed in the study: (a) Dust loading of endotoxin vs. Gram-negative bacteria; (b) Airborne concentration of endotoxin vs. Gram-negative bacteria; (c) Dust concentration of muramic acid vs. Gram-positive bacteria; (d) Dust loading of muramic acid vs. Gram-positive bacteria; (e) Number of occupants vs. Gram-positive bacteria in air; (f) Relative humidity vs. dust concentration of Gram-negative bacteria.

Table 1

Primer sequences for both Gram-positive and Gram-negative bacteria and probe sequences, with their respective reporter and quencher dyes, for each assay (Kärkkäinen et al., 2010). Assays for Gram-positive and Gram-negative bacteria were run in separate wells.

Primers/probes	Sequence (5′-3′)
Forward primer	GGGTTAAGTCCCGCAACGA
Reverse primer	CATTGTAGCACGTGTGTAGCCC
Gram-positive probe	FAM – AAATCATCATGCCCCTTAT – MGBNFQ
Gram-negative probe	FAM – TGACGTCAAGTCATCATGGCCCTTACG – TAMRA

Table 2a

Geometric mean concentrations of Gram-positive and Gram-negative bacterial populations in low (<5) Environmental Relative Moldiness Index (ERMI) homes and high (>5) ERMI homes considering average ERMI values of two years. Significantly different averages are bolded.

Category	Gram-positive bacteria			Gram-negative bacteria		
	Low ERMI homes	High ERMI homes	p-value for t-test	Low ERMI homes	High ERMI homes	p-value for t-test
Dust concentration						
Number of cell equivalents/mg dust	0.5×10^6	1.3×10^6	**<0.001**	1.2×10^5	2.3×10^5	0.196
Dust loading						
Number of cell equivalents/m² floor	2.7×10^8	4.3×10^8	**0.003**	6.0×10^7	7.8×10^7	0.671
Air concentration						
Number of cell equivalents /m³ air	1.4×10^5	1.1×10^5	0.467	3.8×10^3	4.2×10^3	0.750
Number of CFU/m³ air*	8.6×10^2	7.7×10^2	0.806	4	7	0.30

*
Culturable bacteria were analyzed only in air samples and quantified as colony forming units (CFU).

Note: Average amounts of dust collected from low ERMI and high ERMI homes were 1595 mg and 2158 mg, respectively.

Table 2b

Geometric mean concentrations of Gram-positive and Gram-negative bacterial populations in low (<5) Environmental Relative Moldiness Index (ERMI) homes (n = 31) and high (>5) ERMI homes (n = 11) considering ERMI values of 2011. Significantly different averages are bolded.

| Category | Gram-positive bacteria | | | Gram-negative bacteria | | |
	Low ERMI homes	High ERMI homes	p-value for t-test	Low ERMI homes	High ERMI homes	p-value for t-test
Dust concentration						
Number of cell equivalents/mg dust	6.68×10^5	1.08×10^6	0.531	1.34×10^5	2.27×10^5	0.367
Dust loading						
Number of cell equivalents/m^2 floor	3.31×10^8	3.17×10^8	0.953	6.66×10^7	6.69×10^7	0.995
Air concentration						
Number of cell equivalents /m^3 air	1.33×10^5	1.37×10^5	0.915	$\mathbf{3.28 \times 10^3}$	$\mathbf{7.26 \times 10^3}$	**<0.001**
Number of CFU/m^3 air*	4.8×10^2	4.7×10^2	0.952	2	3	0.413

*
Culturable bacteria were analyzed only in air samples and quantified as colony forming units (CFU).

Note: Average amounts of dust collected from low ERMI and high ERMI homes were 1909 mg and 1578 mg, respectively.

Table 3a

Comparison of the geometric mean concentrations of endotoxin and muramic acid in dust and air samples from low (<5) Environmental Relative Moldiness Index (ERMI) homes and high (>5) ERMI homes considering average ERMI values of two years. Significantly different averages are bolded.

Cell wall material	Low ERMI homes	High ERMI homes	p-value for t-test
Endotoxin concentration (EU/mg dust)	**167**	**290**	**0.03**
Endotoxin loading (EU/m^2 floor)	1.0×10^5	0.98×10^5	0.92
Endotoxin air concentration (EU/m^3 air)	5.7	4.8	0.57
Muramic acid concentration (ng/mg dust)	7.0	9.2	0.58
Muramic acid loading (ng/m^2 floor)	3.6×10^3	3.1×10^3	0.80
Muramic acid air concentration (ng/m^3 air)	0.4	0.4	0.90

Table 3b

Comparison of the geometric mean concentrations of endotoxin and muramic acid in dust and air samples from low (<5) Environmental Relative Moldiness Index (ERMI) homes (n = 31) and high (>5) ERMI homes (n = 11) considering ERMI values of 2011. Significantly different averages are bolded.

Cell wall material	Low ERMI homes	High ERMI homes	p-value for t-test
Endotoxin concentration (EU/mg dust)	211.72	203.59	0.897
Endotoxin loading (EU/m^2 floor)	1.07×10^5	0.84×10^5	0.667
Endotoxin air concentration (EU/m^3 air)	**4.44**	**8.64**	**0.026**
Muramic acid concentration (ng/mg dust)	**5.48**	**21.24**	**0.019**
Muramic acid loading (ng/m^2 floor)	2.72×10^3	6.25×10^3	0.187
Muramic acid air concentration (ng/m^3 air)	0.40	0.57	0.336

Table 4

Analysis of the correlations between bacterial measures (geometric mean concentrations) in dust and air as determined by QPCR for samples from all homes. Significant correlation values are bolded.

A. Gram-negative bacteria			
Bacterial measures in dust and air		**Gram-negative dust concentration (number of cell equivalents /mg dust)**	**Gram-negative dust loading (number of cell equivalents /m² floor)**
Gram-negative dust loading (number of cell equivalents /m² floor)	Spearman's rho p value (n)	**.703** **<.001** **(42)**	Not applicable
Gram-negative in air (number of cell equivalents /m³ air)	Spearman's rho p value (n)	.0001 .998 (38)	.096 .568 (38)

B. Gram-positive bacteria			
Bacterial measures in dust and air		**Gram-positive dust concentration (number of cell equivalents /mg dust)**	**Gram-positive dust loading (number of cell equivalents /m² floor)**
Gram-positive dust loading (number of cell equivalents /m² floor)	Spearman's rho p value (n)	**.581** **<.001** **(42)**	Not applicable
Gram-positive in air (number of cell equivalents /m³ air)	Spearman's rho p value (n)	.198 .234 (38)	.231 .163 (38)

Table 5

Analysis of the correlation between bacterial measures (geometric mean concentrations) as determined by QPCR and measures of cell components for samples from all homes. Significant correlation values are bolded.

A. Gram-negative bacteria

Bacterial measures in dust and air		Endotoxin dust concentration (EU/mg dust)	Endotoxin dust loading (EU/m² floor)	Endotoxin in air (EU/m³ air)
Gram-negative dust concentration (number of cell equivalents /mg dust)	Spearman's rho p value (n)	.107 .506 (41)	−.011 .948 (41)	.002 .990 (42)
Gram-negative dust loading (number of cell equivalents /m² floor)	Spearman's rho p value (n)	.002 .988 (41)	**.487** **.001** **(41)**	.057 .720 (42)
Gram-negative in air (number of cell equivalents /m³ air)	Spearman's rho p value (n)	−.059 .731 (37)	.165 .329 (37)	**.594** **<.001** **(38)**

B. Gram-positive bacteria

Bacterial measures in dust and air		Muramic acid dust concentration (ng/mg dust)	Muramic acid dust loading (ng/m² floor)	Muramic acid in air (ng/m³ air)
Gram-positive dust concentration (number of cell equivalents /mg dust)	Spearman's rho p value (n)	**.528** **<.001** **(42)**	.245 .118 (42)	−.150 .349 (41)
Gram-positive dust loading (number of cell equivalents /m² floor)	Spearman's rho p value (n)	.202 .200 (42)	**.434** **.004** **(42)**	−.021 .896 (41)
Gram-positive in air (number of cell equivalents /m³ air)	Spearman's rho p value (n)	.061 .718 (38)	.216 .192 (38)	.243 .147 (37)

Table 6

Correlations between the selected home environmental factors (temperature, relative humidity, number of occupants, age of the home) and geometric mean concentrations of bacteria, endotoxin, and muramic acid. Significant correlation values are bolded.

A. Gram-negative bacteria

Environmental factors/Bacterial measures in dust and air		Gram-negative dust concentration (number of cell equivalents /mg dust)	Endotoxin dust concentration (EU/mg dust)	Gram-negative in air (number of cell equivalents/m³ air)	Culturable Gram-negative in air (CFU/m³ air)	Endotoxin in air (EU/m³ air)
Temperature (°C)	Spearman's rho	.136	.003	−.001	**0.565**	.170
	p value	.390	.985	.996	**<0.001**	.281
	(n)	(42)	(41)	(38)	**(42)**	(42)
Relative humidity (%)	Spearman's rho	**.316**	.067	.035	**0.518**	.194
	p value	**.041**	.677	.837	**<0.001**	.219
	(n)	**(42)**	(41)	(38)	**(42)**	(42)
Occupants (number)	Spearman's rho	.294	.036	−.167	0.125	−.037
	p value	.059	.825	.315	0.432	.814
	(n)	(42)	(41)	(38)	(42)	(42)
Home age (yr.)	Spearman's rho	.184	**.440**	−.076	−0.020	.087
	p value	.254	**.005**	.655	0.904	.591
	(n)	(40)	**(39)**	(36)	(42)	(40)

B. Gram-positive bacteria

Environmental factors / Bacterial measures in dust and air		Gram-positive dust concentration (number of cell equivalents /mg dust)	Muramic acid dust concentration (ng/mg dust)	Gram positive in air (number of cell equivalents/m³ air)	Culturable Gram positive in air (CFU /m³ air)	Muramic acid in air (ng/m³ air)
Temperature (°C)	Spearman's rho	**−.364**	**−.332**	−.219	**0.429**	.221
	p value	**.018**	**.032**	.187	**0.005**	.165
	(n)	**(42)**	**(42)**	(38)	**(41)**	(41)
Relative humidity (%)	Spearman's rho	−.221	.016	−.249	**0.339**	.243
	p value	.159	.919	.131	**0.030**	.147
	(n)	(42)	(42)	(38)	**(41)**	(37)
Occupants (number)	Spearman's rho	.029	**−.353**	**.393**	0.178	.026
	p value	.855	**.022**	**.015**	0.266	.870
	(n)	(42)	**(42)**	**(38)**	(41)	(41)
Home age (yr.)	Spearman's rho	−.144	−.214	−.142	0.034	.054
	p value	.377	.183	.408	0.836	.740
	(n)	(40)	(40)	(36)	(41)	(39)