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## ***Stenotrophomonas*, *Mycobacterium*, and *Streptomyces* in home dust and air: Associations with moldiness and other home/family characteristics**

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### **Abstract**

Respiratory illnesses have been linked to children's exposures to water-damaged homes. Therefore, understanding the microbiome in water-damaged homes is critical to preventing these illnesses. Few studies have quantified bacterial contamination, especially specific species, in water-damaged homes. We collected air and dust samples in twenty-one low-mold homes and twenty-one high-mold homes. The concentrations of three bacteria/genera, *Stenotrophomonas maltophilia*, *Streptomyces* sp. and *Mycobacterium* sp., were measured in air and dust samples using quantitative PCR (QPCR). The concentrations of the bacteria measured in the air samples were not associated with any specific home characteristic based on multiple regression models. However, higher concentrations of *S. maltophilia* in the dust samples were associated with water damage, i.e. with higher floor surface moisture and higher concentrations of moisture-related mold species. The concentrations of *Streptomyces* and *Mycobacterium* sp. had similar patterns and may be partially determined by human and animal occupants and outdoor sources of these bacteria.

### **Keywords**

Air; Dust; Bacteria; Mold; Water damage; Microbiome

### **INTRODUCTION**

Bacterial growth in water-damaged buildings could potentially lead to health problems, e.g., respiratory infections (Thrasher and Crawley, 2009) and atopic and/or non-atopic inflammatory diseases (Douwes et al., 2003). Bacterial contamination in homes may also be a confounder to our understanding of the role of mold contamination in respiratory health. Therefore, contemporaneous quantification of bacterial and mold contamination would be useful to understanding the home and human microbiomes and their interactions.

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Gram-positive and Gram-negative bacteria have been isolated from water-damaged, moldy building materials (Andersson et al., 1997; Peltola et al., 2001; Suihko et al., 2009; Torvinen et al., 2006). Also, a large variety of bacteria have been detected in settled dust from both moisture-damaged and undamaged buildings (Andersson et al., 1997; Rintala et al., 2002; Rintala, 2011). The identification and quantification of bacteria, specifically those associated with water-damaged homes, is needed in order to understand the effect of indoor bacterial contamination on human health. We have focused on three bacteria/genera: *Stenotrophomonas maltophilia*, *Streptomyces*, and *Mycobacterium*.

*Stenotrophomonas maltophilia* is a Gram-negative bacterium (formerly known as *Xanthomonas maltophilia*) which has emerged as an important nosocomial pathogen associated with crude mortality rates ranging from 14 to 69% in patients with bacteremia (Jang et al., 1992; Victor et al., 1994). *S. maltophilia* has been isolated from many environments, including hospitals, the plant rhizosphere, vertebrates and invertebrates, and water [critically reviewed by Brooke, 2012]. Although *S. maltophilia* has been isolated from numerous environmental sources, *S. maltophilia* has never been assessed in the air or dust in home environments.

*Streptomyces* is a common and widespread genus of Gram-positive, spore forming, soil bacterium which can thrive in moist environments. For example, *Streptomyces griseus* and *S. coelicolor* have been isolated from moisture-damaged building materials (Rintala et al., 2002; Suihko et al., 2009). This may be important because some species of *Streptomyces* have been reported to be potent inducers of inflammatory reactions (Huttunen et al., 2003; Jussila et al., 1999) and detection of *Streptomyces* DNA has been inversely associated with the pulmonary function of school children (Simoni et al., 2011). Although *Streptomyces* have previously been quantified in house dust (Johansson et al., 2011; Lignell et al., 2008; Rintala and Nevalainen, 2006) and classroom dust (Simoni et al., 2011), to our knowledge, their concentration has not been measured in home air samples.

*Mycobacterium* is a genus of Gram-positive bacteria previously isolated from water-damaged building materials (Andersson et al., 1997; Torvinen et al., 2006). In addition, *Mycobacterium* cells were found in aerosols generated in the process of dismantling moisture-damaged structures (Rautiala et al., 2004), but their prevalence in indoor air is not known.

In this study, the levels of *S. maltophilia*, *Streptomyces*, and *Mycobacterium* in house dust and air samples were assessed using quantitative PCR analysis (QPCR). These concentrations were evaluated in relationship to the homes' moldiness, as described by the Environmental Relative Moldiness Index (ERMI), and other family/home characteristics.

## MATERIALS AND METHODS

### Homes recruited in the study

The study homes were selected among homes of families participating in the Cincinnati Children's Allergy and Air Pollution Study (CCAAPS) (LeMasters et al., 2006). A group of 42 homes was chosen; 21 homes had ERMI values  $\geq 5.2$  and 21 had ERMI values  $< 5.2$ . This cut-point was selected because we have previously shown that relative moldiness index  $\geq 5.2$  was predictive of asthma development in children (Reponen et al., 2011).

### On-site home visit, sampling, and recording home characteristics

On-site home visits were performed by two-person teams. Information was collected on the following home characteristics: homeowner-reported visible mold, homeowner-reported water damage, dog ownership, and the flooring type in the child's primary activity room

(PAR) (Reponen et al., 2010). The inspection team measured temperature, relative humidity, and floor surface moisture (SurveyMaster Protimeter, General Electric Company, Billerica, MA) in the child's PAR.

Floor dust samples were obtained from all 42 homes for assessment of bacteria and mold in the child's PAR, as described by Cho et al. (2006). Dust samples were collected with a vacuum cleaner (Filter Queen Majestic®; HMI Industries Inc., Seven Hills, Ohio) 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  $\mu\text{m}$  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  $\text{m}^2$  in the middle of the room at a vacuuming rate of 2  $\text{min}/\text{m}^2$ . For non-carpeted floor (hard wood, linoleum, tile, or sheet floor), the entire room floor was vacuumed at a rate of 1  $\text{min}/\text{m}^2$ . Large dust particles were removed by sieving (355- $\mu\text{m}$  mesh sieve), and the resulting dust (particles <355  $\mu\text{m}$  in diameter) was stored at  $-20^\circ\text{C}$  before analyses.

Air samples were collected from 38 of the 42 homes at 3.5 L/min over a 24-hr period using a NIOSH-developed 2-stage cyclone sampler, which classifies airborne particles in three size fractions: <1.0  $\mu\text{m}$ , 1.0–1.8  $\mu\text{m}$ , and >1.8  $\mu\text{m}$  (Lindsley et al., 2006). The cyclone sampler is designed to collect the submicrometer fraction on a polycarbonate filter (Millipore, Billerica, MA), whereas the 1.0–1.8  $\mu\text{m}$  and >1.8  $\mu\text{m}$  size fractions are collected directly into 1.5 mL microcentrifuge tubes.

#### DNA extraction from environmental samples and QPCR analysis

Each dust sample ( $5.0 \pm 0.1$  mg) was extracted by placing the sample in a “bead-beating” tube with glass beads and shaken for 1 min, as previously described (Haugland et al., 2004). The DNA was purified using the DNA-EZ extraction kit (GeneRite, Cherry Hill, NJ). For air samples, the collection tubes of the size fractions 1.0–1.8  $\mu\text{m}$  and >1.8  $\mu\text{m}$  from the NIOSH sampler were used directly as “bead-beating” tubes for the DNA extraction. The polycarbonate filter from the size fraction of <1.0  $\mu\text{m}$  was placed in its own bead-beating tube. DNA from the two stage sampler tubes and from the filter was extracted and purified exactly like the dust samples, described above. Every sample was spiked with 10  $\mu\text{L}$  of a  $2 \times 10^8$  conidia/mL reference suspension of *Geotrichum candidum* as an internal control to ensure that the extraction, purification, and amplification processes were not affected or inhibited. The threshold cycle ( $C_t$ ) value for this internal control had to be within  $\pm 1.5 C_{tS}$  (considered to within the 50% efficiency of extraction and a widely accepted standard for QPCR analysis of environmental samples) for the analysis to be considered accurate. Any sample with an internal control value outside that range was repeated.

The sequences for the *S. maltophilia*, *Streptomyces*, and *Mycobacterium* assays were published previously and are shown in Table 1. The QPCR assays targeted the 16s RNA gene in *S. maltophilia* and *Mycobacterium* (genus) and the 23s RNA gene in *Streptomyces* (genus). Rintala and Nevalainen (2006) chose the 23s RNA gene as the target gene because it was not possible to find a primer/probe set targeting the 16s RNA gene that met the criteria of suitability for QPCR assay and specificity at the same time. For the mold analyses, all primer and probe sequences used in the assays can be found online (US Environmental Protection Agency, 2012). Primers and probes were synthesized commercially (Applied Biosystems, Inc.). Each QPCR reaction contained 12.5  $\mu\text{L}$  of “Universal Master Mix” (Applied Biosystems Inc., Foster City, CA), 1  $\mu\text{L}$  of a mixture of forward and reverse primers at 25  $\mu\text{M}$  each, 2.5  $\mu\text{L}$  of a 400 nM TaqMan probe (Applied Biosystems Inc.), 2.5  $\mu\text{L}$  of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO), 1.5  $\mu\text{L}$  of DNA free water (Cepheid, Sunnyvale, CA), and 5  $\mu\text{L}$  of DNA extract from the sample. Assays were performed using the Roche LightCycler® 480 System (Roche

Applied Science, Indianapolis, IN). Standard curves for each bacteria/genus were generated from pure cultures of *S. maltophilia* (ATCC 13637, American Type Culture Collection, Manassas, VA), *M. intracellulare* (ATCC 700663), and *S. anulatus* (ATCC 27416) and were ultimately based on hemocytometer counts of cells in the highest concentration in the standard curve. DNA extracted from this highest concentration (as previously described) was used to generate a dilution series for the standard curve. Since the QPCR is based on the cells counted in the hemocytometer as the standard, copy number differences are accounted for because the results are expressed as number of cells. The efficiency of amplification calculated from the standard curves was 88% for *S. maltophilia*, 90% for *Mycobacterium*, and 79% for *Streptomyces* (genus). Detection limits, defined at a  $C_t$  value of 40, were 32 cells for *S. maltophilia*, 7 cells for *Mycobacterium* (genus), and 4 cells for *Streptomyces* (genus).

Bacterial dust concentrations were expressed as cell equivalents per milligram dust (cell eq./mg). Bacterial dust loading, expressed as cell equivalents per m<sup>2</sup> floor area (cell eq./m<sup>2</sup>), was derived from concentration by multiplying concentration with total mass of dust vacuumed and dividing by m<sup>2</sup> floor area vacuumed. Bacterial air concentrations were expressed as cell equivalents per m<sup>3</sup> of air sampled (cell eq./m<sup>3</sup>).

The quantification of the mold contamination was based on the QPCR analysis of 36 molds (Vesper et al., 2007). These 36 molds include 26 Group 1 molds that indicate water-damage and 10 Group 2 species that are commonly found, even without water damage. The ERMI values were calculated as shown in Equation Eq. 1, by taking the Sum of the Logs of the concentrations of the Group 1 molds ( $s_1$ ) and subtracting the Sum of the Logs of the concentrations of Group 2 molds ( $s_2$ ).

$$ERMI = \sum_{i=1}^{26} \log_{10}(s_{1j}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \quad (\text{Eq.1})$$

The ERMI scale ranges from approximately -10 to 20 (low to high) and ERMI values can be even higher in highly contaminated homes. The upper quartile (highest mold contamination quartile) starts at an ERMI value of approximately 5 (Vesper et al., 2007).

### Statistical analyses

Dust borne and airborne bacterial concentrations were log-transformed (natural log) to generate normally distributed dependent variable data sets, which were verified using the Kolmogorov-Smirnov test. The three size fractions of airborne bacteria collected with the NIOSH cyclone were initially assayed separately, but then pooled to obtain normally distributed data. Procedurally, non-detections in the air samples were set at half the lower detection limit. An outlier in the *S. maltophilia* dust concentrations was identified using SPSS and removed prior to subsequent analysis.

The association between log<sub>e</sub>-transformed values of each dependent variable and each home characteristic (independent variable) was evaluated by univariate regression. Linearly modeled home characteristics included number of inhabitants, relative humidity, temperature, and the age of home. All remaining home characteristics were modeled categorically. Model building was performed separately for each dependent variable. Final multivariate models were obtained by sequential analyses using a backward stepwise regression with a forward approach. All univariately significant independent variables ( $p < 0.20$ ) 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. The final multivariate model included all independent variables having  $p < 0.05$ .

Pearson product moment correlations were calculated between (1)  $\log_e$ -transformed bacteria dust concentration and ERMI values, (2)  $\log_e$ -transformed dust concentration and air concentration for each bacterial group, and (3)  $\log_e$ -transformed dust loading and air concentration for each bacterial group. Statistical analyses were performed using SPSS Statistics 17.0 and SAS software.

## RESULTS

### Bacterial concentrations in dust

The geometric means of bacterial concentrations in floor dust were 2,170 cell equivalents/mg, 1,905 cell eq./mg, and 69,750 cell eq./mg for *S. maltophilia*, *Streptomyces*, and *Mycobacterium*, respectively. The respective geometric means of floor dust loadings were  $9.35 \times 10^5$  cell eq./m<sup>2</sup>,  $8.47 \times 10^5$  cell eq./m<sup>2</sup>, and  $3.02 \times 10^7$  cell eq./m<sup>2</sup> for all homes sampled.

### Correlation between ERMI and the concentrations of bacteria in dust

The ERMI values ranged from -4.5 to 26.9 with an average value of 3.1 for all homes in this study. As shown in Table 2, the correlation between *S. maltophilia* and ERMI was statistically significant. After parsing ERMI into its Group 1 and Group 2 components, Pearson correlation analysis showed that it is the Group 1 (water-damage) molds that were associated with the higher concentrations of *S. maltophilia* and *Streptomyces* and not the Group 2 (outdoor) molds (Table 2). No statistically significant correlations were found between *Mycobacterium* and ERMI.

### Bacterial concentrations in indoor air

The NIOSH 2-stage cyclone sampler partitioned indoor airborne particles into three size fractions, which were assayed separately for the three target bacteria. Airborne concentrations of *S. maltophilia*, *Streptomyces*, and *Mycobacterium* were all significantly higher in the  $>1.8 \mu\text{m}$  size fraction compared to the other fractions (Table 3). The bacterial concentrations in each of the three size fractions were pooled to determine the overall mean airborne concentrations inside the homes. The geometric mean concentration of airborne *S. maltophilia* was 833 cell equivalents (eq.) /m<sup>3</sup>. For *Streptomyces*, the geometric mean of airborne concentration inside the home was 50 cell eq./m<sup>3</sup>. Highest concentrations were measured for *Mycobacterium*, with a geometric mean airborne concentration of 1702 cell eq./m<sup>3</sup> for all homes sampled.

### Correlations between bacterial concentrations in air and dust

For both *S. maltophilia* and *Mycobacterium*, total airborne concentration was weakly, although significantly, correlated with dust concentration. As shown in Figure 1, the Pearson product moment correlation coefficient between  $\log_e$ -transformed total airborne concentration and dust concentration was strongest for *Mycobacterium*. In general, correlations with total airborne concentrations were lower when dust concentration was converted to “dust loading,” which is the amount of a given bacteria per unit floor area. *Mycobacterium* was the only target bacterial group that had a significant correlation between  $\log_e$ -transformed total airborne concentration and dust loading.

### Univariate analysis on the concentration of bacteria in dust and air as a function of home/family characteristics

The concentration and load of each of the bacteria in the dust samples (unadjusted geometric means) was assessed in terms of home/family characteristics (Table 4). An increase in relative humidity, the age of the home and surface moisture as well as the absence of carpeting were all associated with significantly higher *S. maltophilia* concentrations, but not with dust loads.

Presence of dogs and visible mold were both associated with significantly higher *Streptomyces* concentrations, but not with dust loads. Higher *Streptomyces* dust load was most strongly associated with carpeted floors. Similar to *Streptomyces*, presence of dogs and visible mold were both associated with significantly higher *Mycobacterium* concentrations, but not with dust loads. Lower temperatures were associated with higher *Mycobacterium* concentrations. Higher *Mycobacterium* dust load was most strongly associated with carpeted floors.

The concentration of each of the bacteria in the air samples (unadjusted geometric means) was assessed in terms of home/family characteristics (Table 5). Increased number of occupants was significantly associated with increased *Mycobacterium* concentrations. No statistically significant associations were found between *S. maltophilia* or *Streptomyces* concentrations in the air samples and home/family characteristics.

### Multiple regression models for determinants of bacterial concentrations in dust and air

Home characteristics that were significantly associated with a bacterial concentration in the multiple regression models, along with the non-significant home characteristic with the lowest p-value, are presented in Table 6. Surface moisture remained in the model as a strong determinant of *S. maltophilia* concentration in dust. Similar to univariate analysis, the presence of dog(s) was a strong predictor for an increase in both *Streptomyces* and *Mycobacterium* dust concentrations. None of the multiple regression models for bacterial dust loading and for airborne bacterial levels showed significant associations with the home/family characteristics investigated.

## DISCUSSION

The impact of the home microbiome on the development of the human microbiome and the resulting effects on human health is not well understood. The variability of the home environment as well as the great diversity of microorganisms in the home are the sources of this complexity. Sampling of the air provides only a short glimpse of the home microbiome whereas dust can accumulate microorganisms for an extended period of time. As a result, air and dust samples each provide insights into this dynamic process of microbiome exchanges.

In this study, the air concentrations of *Stenotrophomonas maltophilia*, *Streptomyces* sp. and *Mycobacterium* sp. were not correlated with factors such as reported water damage, visible mold, mold contamination (as describe by the ERMI metric), relative humidity or surface moisture, temperature, dogs, type of flooring or the home's age. Significantly higher *Mycobacterium* concentrations were measured in the air samples of homes with more occupants. However, this association lost significance after adjusting for other home characteristics.

In this study, significantly higher dust concentrations of *Stenotrophomonas maltophilia* were associated with water damage indicators, i.e., surface moisture, ERMI values and Group 1 molds. However, *Streptomyces* sp. and *Mycobacterium* sp. concentrations in dust were significantly associated only with the presence of dogs in the home. Therefore the ecological

niches/sources in homes of *S. maltophilia* cells may be different from those of *Streptomyces* sp. and *Mycobacterium* sp.

Although *S. maltophilia* has been isolated from many environments, it is often thought of as a waterborne bacterium (Brooke, 2012). Our results suggest that a significant, and unexpected, reservoir is water-damaged homes. This discovery may have important implications for human health.

*S. maltophilia* is presently recognized as an emerging global opportunistic pathogen (Brooke 2012). In addition, a few studies of the non-infectious health effects of this bacterium have been published. *S. maltophilia* was found more frequently (based on 16S rRNA analysis) on the skin of patients having atopic dermatitis compared to the controls (Dekio et al., 2007). In another clinical observation, an asthmatic adult with allergic bronchopulmonary aspergillosis was co-infected with *S. maltophilia* (McCallum et al., 2005). *S. maltophilia* was more frequently isolated from the sputum of cystic fibrosis patients with allergic bronchopulmonary aspergillosis than from controls (Ritz et al., 2005). This bacterium has also been isolated from sinus cultures in refractory chronic rhinosinusitis (Grindler et al., 2010). Since it appears that this bacterium is associated with water-damaged homes, we need to learn much more about its role in non-infectious respiratory health.

Similar to *S. maltophilia*, some *Mycobacterium* and *Streptomyces* species are human pathogens (Thrasher and Crawley, 2009) but also have health effects that are not based on infections (Cai et al., 2011; Lappalainen et al. 2012). These bacteria have been previously isolated from moisture-damaged building materials (Suihko et al., 2009; Torvinen et al., 2006). However, in our study, the concentrations of these genera were not correlated with high ERMI homes, even though the geometric means of *Streptomyces* and *Mycobacterium* concentrations in our dust samples were higher than means reported in earlier studies (Lignell et al., 2008; Rintala and Nevalainen, 2006; Simoni et al., 2011; Torvinen et al., 2010). These variations might have occurred, in part, due to differences in the sampling as well as in the dust processing methodologies, as discussed for *Streptomyces* in Johansson et al. (2011).

The NIOSH-sampler collects particles into three different size fractions, but we found most of these bacteria in the fraction of  $> 1.8 \mu\text{m}$ . At first this may seem surprising since *S. maltophilia* is a rod-shaped bacterium with length of 0.5-1.5  $\mu\text{m}$  and width of 0.4-0.7  $\mu\text{m}$  (Adamek and Bathe, 2011; de Oliveira-Garcia et al., 2002) and *Mycobacterium* has rod-shaped vegetative cells with characteristic aerodynamic diameters ranging from  $\sim 0.7$ -2.0  $\mu\text{m}$  (McCullough et al., 1997; Peccia et al., 2007; Schafer et al., 1998). Also, *Streptomyces* grows as a thread-like network of cells from which spore chains of varying length develop. These spores typically have an aerodynamic diameter  $\sim 1.0 \mu\text{m}$  (Madelin and Johnson, 1992; Reponen et al., 1996). Therefore, it appears that the airborne cells of these bacteria are primarily in aggregates  $> 1.8 \mu\text{m}$ . These aggregated particles may be more likely to become airborne compared to individual cells and cause inhalation exposure because a significant fraction of the aggregated cells could be within the inhalable particle size range.

We found that total airborne concentrations of *S. maltophilia* and *Mycobacterium* were weakly, although significantly, correlated with dust concentration. The strongest correlation between airborne and dust borne bacterial concentration was for *Mycobacterium* ( $r = 0.454$ ) which could be due in part to having fewer air samples below the limit of detection compared to the *S. maltophilia* and *Streptomyces* assays. Bacterial dust load, which is the amount of given bacteria per unit area, may be a useful measure of contaminant levels because it represents the total burden of the bacteria in the home (Johansson et al., 2011). However, in our study, more significant associations with home characteristics and airborne

concentrations were found for dust concentrations than for loads. This may be due to a larger coefficient of variation in the load measurements compared to that in the concentration measurements.

Based on multiple regression models, surface moisture remained significantly associated with *S. maltophilia* concentrations in dust. However, dust concentrations of the *Streptomyces* and *Mycobacterium* genera were significantly higher in homes with one or more dogs and a weak association was also seen between airborne *Mycobacterium* and number of occupants. We suggest that some of *Streptomyces* and *Mycobacterium* cells measured in the house dust could have been transferred from outdoors to the indoor environment carried by human and animal foot traffic. This association between *Streptomyces* dust concentration and dog ownership is consistent with Johansson et al. (2011).

In conclusion, *S. maltophilia* concentrations in indoor dust were associated with higher ERMI and ERMI Group 1 mold species and with floor surface moisture. The biological significance of the increased exposure to *S. maltophilia* in homes with high ERMI values requires further study.

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### NOTICE

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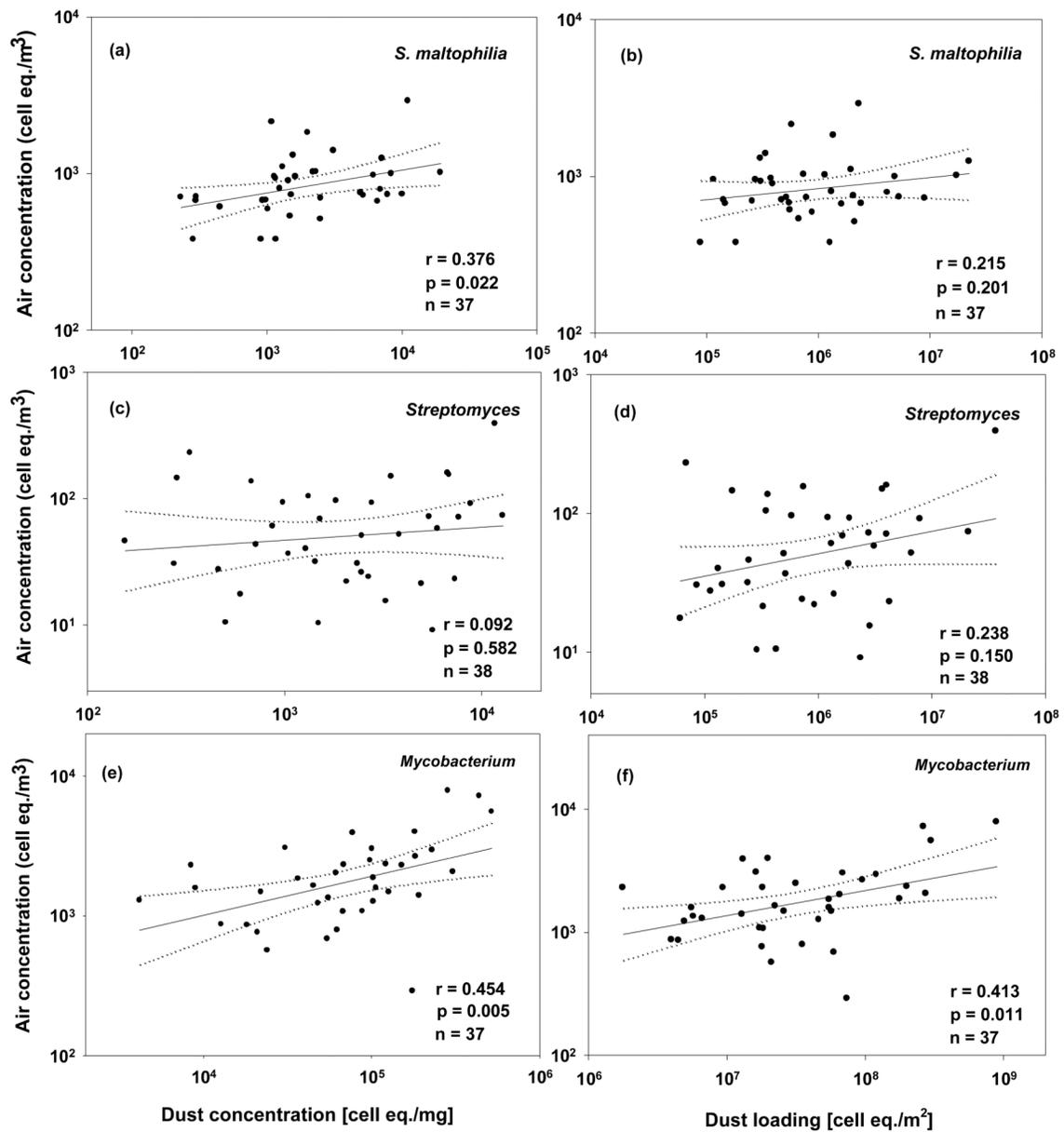
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### Practical Implications

Since *S. maltophilia* is a known respiratory pathogen, its concentrations in homes should be monitored to better understand its health implications, especially as a co-contaminant with mold.



**Figure 1.** Correlations between air concentration and dust concentration (a, c, e) and between air concentration and dust loading (b, d, f) for the three bacteria/genera. Dotted lines are 95% confidence lines. Correlation coefficients are based on Pearson product moment correlations between log-transformed outcome variables.

**Table 1**

Primers and probes with their respective reporter dye and quencher. A standardized qPCR program was used for all three primer sets consisting of an initial incubation step at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10s and annealing/extension at 60°C for 30s.

Target bacteria		Sequence (5 -3 )
<i>S. maltophilia</i>	Forward primer	GATCCTGGCTCAGAGTGAACG
	Reverse primer	CCCACGACAGAGTAGATTCCG
	Probe	FAM-CACCCGTCCGCCACTCGCCAC-TAMRA
	Reference	Rios-Licea et al. (2010)
<i>Streptomyces</i>	Forward primer	GCCGATTGTGGTGAAGTGGA
	Reverse primer	GTACGGGCCGCCATGAAA
	Probe	FAM-ATCCTATGCTGTCGAGAAAAGCCTCTAGCG-TAMRA
	Reference	Rintala and Nevalainen (2006)
<i>Mycobacterium</i>	Forward primer	GATGCAACGCGAAGAACCTT
	Reverse primer	TGCACCACCTGCACACAGG
	Probe	FAM-CCTGGGTTGACATGCACAGGACG-TAMRA
	Reference	Torvinen et al. (2010)

**Table 2**

Correlations between bacteria concentration in house dust and overall ERMI, Group 1 ERMI, and Group 2 ERMI. Correlation coefficients (r) are based on Pearson product moment correlations between loge-transformed outcome variables. Significant correlations (p<0.05) are shown in bold. Correlations between ERMI (including Group 1 and Group 2) and *Mycobacterium* were not significant.

	<i>S. maltophilia</i>		<i>Streptomyces</i>	
	r	n*	r	n
ERMI	<b>0.341</b>	41	0.298	42
ERMI Group 1	<b>0.364</b>	41	<b>0.308</b>	42
ERMI Group 2	0.184	41	0.157	42

\* One *S. maltophilia* dust concentration was excluded as an outlier.

**Table 3**

Size-fractionated airborne bacteria concentrations. Significant differences between the three size fractions from ANOVA analysis ( $p < 0.05$ ) are shown in bold.

Target bacteria	n	Geometric mean (geometric standard deviation) [cell eq./m <sup>3</sup> ]			p
		< 1.0 $\mu\text{m}$	1.0 – 1.8 $\mu\text{m}$	> 1.8 $\mu\text{m}$	
<i>S. maltophilia</i>	38	201 (1.6)	204 (1.6)	<b>359 (2.0)</b>	< 0.01
<i>Streptomyces</i>	38	21 (2.8)	43 (3.1)	<b>106 (3.5)</b>	< 0.01
<i>Mycobacterium</i>	38	264 (2.1)	298 (1.8)	<b>984 (2.5)</b>	< 0.01

**Table 4**

Home/family characteristics as univariate predictors of bacterial levels in household dust ( $d_p < 355 \mu\text{m}$ ). Significant associations ( $p < 0.05$ ) are shown in bold and underlined.

Home characteristic	n <sup>#</sup>	Dust concentration [cell eq./mg] Geometric mean (geometric standard deviation)			Dust loading [cell eq./m <sup>2</sup> ] Geometric mean (geometric standard deviation)		
		<i>S. maltophilia</i>	<i>Streptomyces</i>	<i>Mycobacterium</i>	<i>S. maltophilia</i>	<i>Streptomyces</i>	<i>Mycobacterium</i>
<i>Direct factors/sources</i>							
Number of inhabitants*							
3	8	3147 (3.7)	2201 (3.6)	6.60 × 10 <sup>4</sup> (3.9)	1.85 × 10 <sup>6</sup> (5.1)	1.30 × 10 <sup>6</sup> (8.1)	3.49 × 10 <sup>7</sup> (8.8)
4	14	2073 (2.2)	2223 (2.2)	8.95 × 10 <sup>4</sup> (2.1)	7.87 × 10 <sup>5</sup> (3.5)	8.44 × 10 <sup>5</sup> (4.2)	3.38 × 10 <sup>7</sup> (3.7)
5	20 <sup>‡</sup>	1920 (3.9)	1614 (3.5)	5.99 × 10 <sup>4</sup> (3.5)	7.95 × 10 <sup>5</sup> (3.9)	7.16 × 10 <sup>5</sup> (4.5)	2.66 × 10 <sup>7</sup> (4.0)
<i>p</i>		0.55	0.22	0.47	0.98	0.77	0.44
Presence of dogs <sup>†</sup>							
No	24	2092 (3.4)	1308 (2.7)	4.53 × 10 <sup>4</sup> (2.8)	9.83 × 10 <sup>5</sup> (3.4)	6.14 × 10 <sup>5</sup> (4.0)	2.03 × 10 <sup>7</sup> (3.7)
Yes	18 <sup>‡</sup>	2286 (3.1)	3144 (2.9)	1.21 × 10 <sup>5</sup> (2.7)	8.71 × 10 <sup>5</sup> (4.8)	1.30 × 10 <sup>6</sup> (5.9)	5.01 × 10 <sup>7</sup> (4.8)
<i>p</i>		0.85	<u><b>&lt;0.01</b></u>	<u><b>&lt;0.01</b></u>	0.78	0.13	0.09
<i>Indirect factors/sources</i>							
Reported water damage <sup>‡</sup>							
No	16	2120 (3.3)	1599 (3.4)	5.75 × 10 <sup>4</sup> (3.6)	7.78 × 10 <sup>5</sup> (3.6)	5.87 × 10 <sup>5</sup> (4.6)	2.11 × 10 <sup>7</sup> (4.3)
Yes	26 <sup>‡</sup>	2203 (3.3)	2121 (2.8)	7.89 × 10 <sup>4</sup> (2.7)	1.05 × 10 <sup>6</sup> (4.3)	1.06 × 10 <sup>6</sup> (5.0)	3.81 × 10 <sup>7</sup> (4.4)
<i>p</i>		0.89	0.41	0.54	0.50	0.36	0.45
Reported visible mold <sup>‡</sup>							
No	19	2110 (3.1)	1371 (3.2)	4.81 × 10 <sup>4</sup> (3.1)	8.46 × 10 <sup>5</sup> (4.1)	5.49 × 10 <sup>5</sup> (5.5)	1.93 × 10 <sup>7</sup> (4.8)
Yes	23 <sup>‡</sup>	2223 (3.4)	2499 (2.8)	9.62 × 10 <sup>4</sup> (2.8)	1.02 × 10 <sup>6</sup> (4.0)	1.21 × 10 <sup>6</sup> (4.1)	4.46 × 10 <sup>7</sup> (3.7)
<i>p</i>		0.93	<u><b>0.05</b></u>	<u><b>0.04</b></u>	0.69	0.08	0.10
Relative humidity(%)*							
21.0-30.4	14	1286 (4.0)	1399 (3.4)	5.93 × 10 <sup>4</sup> (4.0)	6.38 × 10 <sup>5</sup> (4.2)	6.94 × 10 <sup>5</sup> (3.6)	2.74 × 10 <sup>7</sup> (3.9)
30.5-40.9	14 <sup>‡</sup>	2355 (2.6)	2237 (3.2)	7.40 × 10 <sup>4</sup> (3.0)	1.00 × 10 <sup>6</sup> (2.9)	1.05 × 10 <sup>6</sup> (4.5)	3.47 × 10 <sup>7</sup> (3.8)

Home characteristic	n <sup>#</sup>	Dust concentration [cell eq./mg] (geometric standard deviation)			Dust loading [cell eq./m <sup>2</sup> ] (geometric standard deviation)		
		<i>S. maltophilia</i>	<i>Streptomyces</i>	<i>Mycobacterium</i>	<i>S. maltophilia</i>	<i>Streptomyces</i>	<i>Mycobacterium</i>
41.0-57.3	14	3397 (2.7)	2208 (2.6)	7.64 × 10 <sup>4</sup> (2.6)	1.28 × 10 <sup>6</sup> (4.9)	8.34 × 10 <sup>5</sup> (7.3)	2.89 × 10 <sup>7</sup> (5.9)
<i>p</i>		<b>0.05</b>	0.65	0.97	0.39	0.75	0.57
Temperature(°C) <sup>*</sup>							
13.0-21.8	15	2127 (3.3)	2313 (2.9)	9.82 × 10 <sup>4</sup> (2.6)	9.23 × 10 <sup>5</sup> (5.7)	9.61 × 10 <sup>5</sup> (5.3)	4.08 × 10 <sup>7</sup> (4.5)
21.9-25.0	14	1982 (3.0)	2009 (2.8)	8.04 × 10 <sup>4</sup> (3.6)	7.11 × 10 <sup>5</sup> (2.9)	7.21 × 10 <sup>5</sup> (4.3)	2.62 × 10 <sup>7</sup> (4.7)
25.1-27.8	13 <sup>‡</sup>	2397 (3.7)	1438 (3.5)	4.08 × 10 <sup>4</sup> (2.8)	1.34 × 10 <sup>6</sup> (3.4)	8.71 × 10 <sup>5</sup> (5.6)	2.47 × 10 <sup>7</sup> (4.3)
<i>p</i>		0.82	0.07	<b>0.02</b>	0.91	0.40	0.18
Surface moisture <sup>‡</sup>							
Air-dry	33 <sup>‡</sup>	1793 (3.1)	1839 (3.1)	6.94 × 10 <sup>4</sup> (3.1)	9.84 × 10 <sup>5</sup> (4.1)	1.04 × 10 <sup>6</sup> (4.7)	3.84 × 10 <sup>7</sup> (4.1)
Borderline damp	6	6108 (2.9)	2095 (3.7)	8.77 × 10 <sup>4</sup> (4.0)	1.04 × 10 <sup>6</sup> (4.8)	3.57 × 10 <sup>5</sup> (4.8)	1.49 × 10 <sup>7</sup> (5.4)
<i>p</i>		<b>0.02</b>	0.75	0.64	0.93	0.14	0.16
General home characteristics							
Flooring type <sup>‡</sup>							
Hard surface	7	5822 (1.7)	2316 (3.0)	9.70 × 10 <sup>4</sup> (3.7)	6.47 × 10 <sup>5</sup> (3.2)	2.57 × 10 <sup>5</sup> (4.6)	1.08 × 10 <sup>7</sup> (5.5)
Carpet/rug	35 <sup>‡</sup>	1771 (3.2)	1832 (3.1)	6.52 × 10 <sup>4</sup> (3.0)	1.01 × 10 <sup>6</sup> (4.2)	1.07 × 10 <sup>6</sup> (4.5)	3.74 × 10 <sup>7</sup> (3.9)
<i>p</i>		<b>0.01</b>	0.57	0.39	0.45	<b>0.03</b>	<b>0.05</b>
Age of home (yr) <sup>*</sup>							
> 77	13	4040 (3.0)	2833 (2.5)	1.17 × 10 <sup>5</sup> (2.9)	1.94 × 10 <sup>6</sup> (3.8)	1.36 × 10 <sup>6</sup> (5.6)	5.20 × 10 <sup>7</sup> (5.9)
35 - 77	14	2563 (2.8)	1826 (2.8)	7.45 × 10 <sup>4</sup> (2.8)	9.25 × 10 <sup>5</sup> (4.2)	6.59 × 10 <sup>5</sup> (5.0)	2.69 × 10 <sup>7</sup> (4.7)
< 35	13 <sup>‡</sup>	1146 (2.9)	1559 (3.3)	5.43 × 10 <sup>4</sup> (2.6)	4.55 × 10 <sup>5</sup> (3.2)	6.90 × 10 <sup>5</sup> (4.5)	2.41 × 10 <sup>7</sup> (3.1)
<i>p</i>		<b>0.03</b>	0.12	<b>0.03</b>	0.06	0.32	0.31

Note: Covariates that were significant at the 20% level in these univariate regression models were included in the initial multivariate model.

\* Linearly modeled variable. For this table, categorized into approximate tertiles.

‡ Categorical variable.

# Information on some home characteristics was missing and therefore, n does not add up to 42.

<sup>‡</sup>One *S. maltophilia* dust concentration was excluded as an outlier, leaving n - 1 samples for both *S. maltophilia* columns.

**Table 5**

Home/family characteristics as univariate predictors of bacterial levels in air samples. Concentrations have been pooled from all three size ranges. Significant associations ( $p < 0.05$ ) are shown in bold and underlined.

Home characteristic	n <sup>#</sup>	Air concentration [cell eq./m <sup>3</sup> ] Geometric mean (geometric standard deviation)		
		<i>S. maltophilia</i>	<i>Streptomyces</i>	<i>Mycobacterium</i>
<i>Direct factors/sources</i>				
Number of inhabitants *				
3	8	1046 (1.8)	70 (3.7)	1482 (2.8)
4	13	723 (1.4)	37 (2.2)	1334 (1.5)
5	17	833 (1.4)	54 (2.1)	2189 (1.8)
<i>p</i>		0.95	0.55	<b><u>0.01</u></b>
Presence of dogs †				
No	21	791 (1.5)	47 (2.3)	1438 (1.6)
Yes	17	889 (1.6)	54 (2.7)	2097 (2.3)
<i>p</i>		0.41	0.84	0.17
<i>Indirect factors/sources</i>				
Reported water damage †				
No	14	874 (1.6)	38 (2.1)	1663 (1.9)
Yes	24	810 (1.5)	59 (2.6)	1726 (2.1)
<i>p</i>		0.61	0.35	0.88
Reported visible mold †				
No	17	753 (1.7)	49 (2.4)	1502 (1.9)
Yes	21	904 (1.3)	51 (2.5)	1884 (2.1)
<i>p</i>		0.22	0.81	0.33
Relative humidity (%) *				
21.0-30.4	14	806 (1.5)	40 (2.1)	1636 (1.8)
30.5-40.9	13	922 (1.7)	63 (2.8)	1879 (2.2)
41.0-57.3	11	771 (1.4)	51 (2.6)	1593 (2.1)
<i>p</i>		0.97	0.56	0.90
Temperature(°C) *				
13.0-21.8	15	935 (1.5)	47 (2.6)	2188 (1.9)
21.9-25.0	12	723 (1.6)	50 (2.1)	1517 (2.1)
25.1-27.8	11	831 (1.6)	56 (2.9)	1371 (1.9)
<i>p</i>		0.60	0.41	0.18
Surface moisture †				
Air-dry	30	796 (1.5)	50 (2.3)	1676 (1.9)
Borderline damp	5	1171 (1.8)	73 (3.4)	2570 (2.1)
<i>p</i>		0.07	0.39	0.19

Home characteristic	n <sup>#</sup>	Air concentration [cell eq./m <sup>3</sup> ] Geometric mean (geometric standard deviation)		
		<i>S. maltophilia</i>	<i>Streptomyces</i>	<i>Mycobacterium</i>
<i>General home characteristics</i>				
Flooring type <sup>†</sup>				
Hard surface	6	1119 (1.7)	53 (2.8)	2451 (1.8)
Carpet/rug	32	788 (1.5)	50 (2.4)	1590 (2.0)
<i>p</i>		0.07	0.99	0.17
Age of home (yr) <sup>*</sup>				
> 77	11	890 (1.6)	81 (2.6)	2241 (1.9)
35 - 77	12	816 (1.6)	44 (2.2)	1519 (2.2)
< 35	13	836 (1.5)	42 (2.4)	1666 (1.8)
<i>p</i>		0.63	0.12	0.23

Note: Covariates that were significant at the 20% level in these univariate regression models were included in the initial multivariate model.

\* Linearly modeled variable. For this table, categorized into approximate tertiles.

† Categorical variable.

# Information on some home characteristics was missing and therefore, n does not add up to 38.

**Table 6**

Multivariate association between levels of microbial contaminants and home characteristics. Significant associations ( $p < 0.05$ ) are shown in bold.

Exposure (dependent variable)	Target bacteria	Home characteristic (independent variable)	Effect size * (95% CI)
Dust concentration [cell eq./mg]	<i>S. maltophilia</i>	Surface moisture	<b>1.23 (0.21, 2.24)</b>
	<i>Streptomyces</i>	Presence of dog(s)	<b>0.94 (0.29, 1.59)</b>
	<i>Mycobacterium</i>	Presence of dog(s)	<b>0.90 (0.24, 1.55)</b>
Dust loading [cell eq./m <sup>2</sup> ]	<i>S. maltophilia</i>	Age of home	0.51 (-0.01, 1.04)
	<i>Streptomyces</i>	Presence of dog(s)	0.94 (-0.06, 1.95)
	<i>Mycobacterium</i>	Presence of dog(s)	0.84 (-0.10, 1.69)
Air concentration [cell eq./m <sup>3</sup> ]	<i>S. maltophilia</i>	Surface moisture	0.38 (-0.04, 0.81)
	<i>Streptomyces</i>	Age of home	0.29(-0.08, 0.67)
	<i>Mycobacterium</i>	Temperature	-0.69(-1.53, 0.14)

\* Effect size is equal to the change in the dependent variable corresponding to a unit increase (in logescale) in the independent variable. Note: initial multivariate model included covariates which were significant at the 20% level in the univariate regression models.