

Indoor airborne fungi and wheeze in the first year of life among a cohort of infants at risk for asthma

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In studies worldwide, respiratory outcomes such as cough, wheeze and asthma have been consistently linked to mold exposure. Young children spend most of their time indoors and may be particularly vulnerable. We evaluated the associations between exposure to airborne fungal levels and episodes of wheezing in a cohort of 103 infants at risk for asthma (due to maternal history of asthma), living primarily in low-income urban settings. Using a new protocol that facilitates identification of rare and slow-growing fungi, we measured the type and concentration of cultured fungi in home air samples taken early in the infant's first year of life. We also inspected the homes for visible mold, water damage and other housing and environmental conditions. All homes had measurable indoor airborne fungi and 73%, had some sign of mold, water damage, dampness or a musty odor. One or more episodes of wheeze during the first year of life were observed in 38% of infants. Multiple logistic regression showed high indoor levels of *Penicillium* were a significant risk factor for wheeze (OR 6.18; 95% CI: 1.34–28.46) in the first year of life after controlling for season of sampling, smoking, endotoxin levels, day care attendance and confounders. *Acrodictium*, a rarely reported fungal genus, was detected in 18% of study homes, and was associated with wheeze in unadjusted models (OR 2.75; 95% CI 0.99–7.61), but not after adjustment for confounders. Total fungal levels, visually observed mold, dampness, water damage or musty odors were not significantly associated with wheeze.

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Introduction

There is increasing public concern about health effects from mold inhalation. Moldy homes as a consequence of flooding, hurricanes, faulty construction or poor repair have become common in many communities. As much as half of all homes in the United States are estimated to be affected by dampness or mold (Mudarri and Fisk, 2007). A growing number of epidemiologic studies worldwide link fungi and dampness with respiratory symptoms and conditions (Dales et al., 1991; Jaakkola et al., 1993; Bjornsson et al., 1995; Verhoeff

and Burge, 1997; Peat et al., 1998; Dharmage et al., 2001; Bornehag et al., 2001; Zock et al., 2002; Bornehag et al., 2004; Spengler et al., 2004). A National Academy of Sciences review found sufficient evidence of an association between exposure to damp indoor environments and health outcomes including wheeze, cough, upper respiratory symptoms and asthma symptoms in sensitized asthmatic persons (Institute of Medicine, 2004). Recently, a meta-analysis of studies indicated that building dampness and mold are associated with 30–50% increases in respiratory outcomes such as wheeze, cough and asthma (Fisk et al., 2007). Mold and dampness are general terms, and thus the specific agents responsible for health effects are not known. In addition, exposure to mold and dampness has been evaluated in a variety of ways; observation, occupant report and the collection of air or dust samples for spores or fungi are some of the methods that have been utilized. More precise identification of the agents associated with respiratory health

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outcomes is important for increasing our understanding and for eventual prevention and intervention efforts.

Infants and young children are at particular risk for indoor mold and dampness exposure, as they spend a considerable proportion of time in the home environment during a period of intense growth and development of the immunologic and respiratory systems (Martinez et al., 1995; Zeldin et al., 2006). Further, children from lower socioeconomic status households in older inner-city neighborhoods may be at a greater risk due to the age and poor condition of housing stock. This paper summarizes measurable indoor fungal levels and housing characteristics, which promote fungal growth, and wheezing episodes in the first year of life in a cohort of inner city infants at risk for the development of asthma. A newly developed protocol for the analysis of air samples was employed that facilitates growth of rare and slow-growing fungi, thereby enhancing identification of as many species of fungi as possible.

Methods

Study Design and Sample Selection

The AUDIT (Assessment of Urban Dwellings for Indoor Toxics) project was a 2-year study of a cohort of infants at risk for the development of asthma due to a positive maternal history; the design and implementation have been described earlier (Crawford et al., 2006). In brief, mothers with asthma from the low-income urban obstetric population of Syracuse, NY, were recruited between April 2001 and August 2002. Of 370 women identified at 14 recruitment sites, 184 agreed to be contacted. Eligibility requirements included a medically documented history of asthma in the mother, English or Spanish speaking and an expectation of remaining in the home for at least 1 year. Participating mothers were residents of the city of Syracuse or from an adjacent urban location. Eligibility criteria for the baby included: a gestational age of 37 weeks or more, a birth weight of at least 2500 g, the absence of a major congenital malformation and singleton birth.

A total of 135 pregnant mothers met the eligibility criteria, completed consents and were enrolled. Births occurred between June 2001 and September 2002. Following birth, several infants were ineligible due to multiple births ($n = 1$); still birth ($n = 1$); fetal demise ($n = 1$) and pre-term delivery ($n = 4$). In addition, 12 mother–infant pairs moved outside of target area, whereas three others reported that the study was “too much trouble”; eight had insufficient data for analysis. Two infants were placed in foster care and consent for participation was withdrawn by the guardian. The birth cohort is comprised of 103 eligible infants who completed all phases of the study.

Mothers were reimbursed a total of \$100, when in the AUDIT study. Reimbursement was divided over the active

study period of approximately 15 months, to encourage continued participation. The study was reviewed and approved by the State University of New York (SUNY) Upstate Medical University Institutional Review Board.

Clinical Data Collection

Data collection was extensive beginning with an initial interview conducted by a nurse practitioner (NP) during the mother's last trimester of pregnancy. Fluent in both Spanish and English, the NP obtained informed consent, determined the safety and security of the home, and administered a questionnaire detailing the mother's medical history, lifestyle factors, demographics and perceptions of asthma.

Clinical assessments of infants were undertaken at 3, 6, 9 and 12 months of age by the NP; all assessments occurred at the mother's or primary care giver's residence. These visits included collection of the infant's urine sample, a physical exam and the administration of a health questionnaire covering topics such as infant diet, medication use, illnesses, day care, smoking in the home and the presence of pets. Clinical data were also obtained through prenatal and maternal peripartum chart review as well as from the review of the neonatal and pediatric records.

Environmental Sampling

Environmental sampling in the home was undertaken when the infant was approximately 3 months old. A trained field team sampled the air for temperature, humidity, particulate matter, combustion gases, volatile organic compounds and viable fungi and bacteria. Allergen and endotoxin levels were assessed from settled dust samples. Dust samples were collected from the main living room floor of the residence. Dust was obtained using a high volume surface vacuum sampler with a cyclone collector and an autoclaved polypropylene collection bottle. Endotoxin concentrations in dust were determined by the kinetic Limulus assay with the resistant-parallel-line estimation (KLARE) method (Milton et al., 1997). Home visits also included a walk-through inspection to assess the presence of pets, pests, smokers, visible dampness/water, visible mold and a measure of overall home cleanliness. As data collection was extensive, additional findings from both the clinical and the indoor environmental data are summarized in Hunt et al., 2003; Rosenbaum et al., 2005; Hunt et al., 2005 and Crawford et al., 2005.

The bioaerosol sampling protocol was developed and implemented by the SUNY College of Environmental Science and Forestry, in Syracuse, NY. Viable fungi and bacteria were collected using a single-stage Andersen N6 air sampler with impaction for 3 or 6 min onto malt extract agar plates. Samples were hand-delivered to the lab typically within 3 h of collection.

Viable air samples were taken indoors in the main living area and outdoors near the front door at the beginning and

end of the 24-h visit period. Three indoor samples (two sequential samples on day 1 and one on day 2) were obtained while two outdoor samples were collected, one each day. A new subsampling method for analysis of air samples was developed to comprehensively evaluate the microbial population by identifying as many species of airborne fungi as possible. Details of the method and rationale have been described earlier (Catranis et al., 2006). Briefly, each sample consisted of two replicate plates. Plate 1 was handled as a typical Andersen plate; it was left untouched and was used to determine the total colony count. Plate 2 was subsampled to facilitate isolate identification, particularly of rare species and slow-growing fungi. Without subsampling, plates will tend to be dominated by common, fast-growing fungal species, limiting the possibility for detecting slow growers. A random sample of 50 impaction points or plugs from plate 2 were removed and inoculated onto five fresh malt extract agar plates; these were termed Random-50 or R-50 plates. All plates were then incubated at room temperature. Colony-forming units (CFUs) were counted on all plates at 2–3 and 7–10 days after collection. The detection limit for the Andersen 3 min sampling period was <95.2 CFUs, whereas it was <47.6 CFUs for the 6 min period. Concentrations in CFU/m³ were calculated according to the volume of air sampled. For subsampled plates, an additional conversion factor was used to account for the fraction of the plate evaluated. Cultures appearing on the R-50 plates were transferred to slides for microscopic observation and identification to genera and species following the methods of Wang (1990). Total fungal colony counts derived from the subsampling procedure were highly correlated ($R^2=0.92$) with counts obtained using standard procedures (Catranis et al., 2006).

Field blanks (control plates) were taken and handled identically to sample plates except that no air was pumped through the sampler. Three blanks were taken at each home. Colonies occurred rarely in blank plates; nearly all (>95%) of the blank plates had zero growth or one colony, therefore sample counts were not adjusted for blank readings.

Variable Descriptions

A diagnosis of wheeze during the first year of life was defined as: (1) primary care provider-documented wheezing, reactive airway disease, asthma or bronchiolitis or (2) wheeze heard on physical exam by the nurse practitioner or (3) a prescription for bronchodilator, inhaled steroid or steroid pulse prescription documented in the medical record. One or more episodes of wheeze during the first year of life was the dependent variable of interest.

Measurable total fungal levels were present in all 103 homes, with differences in the frequencies of specific taxa observed across homes. Only those taxa observed in at least 15% of homes have been summarized here. These include *Penicillium*, *Cladosporium*, *Aspergillus*, *Basidiomycetes*,

Alternaria, bacteria, yeast, hyaline unknowns (non-sporulating fungi with colorless or light colored hyphae) and dark unknowns (non-sporulating fungi with dark hyphae). A rarely reported fungus, *Acrodontium* (Fernando et al., 2005) also was evaluated in this report.

Mean total indoor fungi, hyaline and dark unknowns, bacteria, yeast and genera-specific fungal levels (e.g., *Penicillium*) were calculated using all three indoor samples collected at the home visit. Values below the detection limit of 95.2 CFUs (3 min sampling) were coded as “2” and those below the detection limit of 47.6 (6 min sampling) were coded as “1” in the mean calculation. As the bacterial and fungal distributions were positively skewed, bioaerosol variables were categorized prior to analysis based on the observed distributions of each. Total indoor fungal levels and the hyaline unknowns, found in more than 85% of homes, were divided into quartiles with the lowest quartile serving as the referent category. For the genus-specific fungi, as well as for yeasts, bacteria and dark unknowns, the distributions were divided into non-detectable (the referent), and “low” and “high” levels. “High” values of *Aspergillus*, *Penicillium*, *Cladosporium*, bacteria, yeast and dark unknowns were defined as those above the 75th percentile. For *Basidiomycetes*, “high” fungal levels were defined as those above the 80th percentile because only 41% of homes had detectable levels. The “low” category was comprised of values below the 75th percentile (*Aspergillus*, *Penicillium*, *Cladosporium*, bacteria, yeast, dark unknowns) or the 80th percentile (*Basidiomycetes*), which were also above the detection limits. *Alternaria* and *Acrodontium* were divided into non-detected and detected because fewer than 20% of homes had measurable levels of fungi identified to these genera (Table 1).

Potential covariables assessed in the analyses included several demographics such as the baby’s gender (female was the referent category); the baby’s race: white (referent) and non-white; Hispanic ethnicity: no (referent) and yes; mother’s educational level: ≤high school, some college or more (referent); insurance: private (referent), Medicaid; mother’s marital status: married (referent), not married; and the mother’s age at the time of delivery (in years). Additional variables with potential associations with wheeze based on the literature were mother’s smoking during pregnancy; any smoking in the home during the first year of life; ever breast-feeding; and attendance at a day care center or non-relative day care home, a surrogate for exposure to respiratory tract infections. Each of these risk factors was dichotomous (yes/no) with “no” as the referent category. Endotoxin levels in EU/mg dust were treated as a dichotomous variable in unadjusted analyses: <100 EU/mg dust (referent) versus ≥100 EU/mg dust and as a continuous variable in fungal models due to the small sample size.

Variables specific to the environmental sampling visit were the child’s age at the home visit (in months); whether the family changed residences over the study period, yes or no

Table 1. Indoor airborne fungi and bacteria levels in AUDIT homes.

Taxa or Genera	Percent homes where detected	High concentration	
		Value CFU/m ³	Description
Total fungi	100	> 1214	75th percentile
Hyaline unknown ^a	86	> 381	75th percentile
Bacteria	77	> 128	75th percentile
<i>Penicillium</i>	74	> 120	75th percentile
<i>Cladosporium</i>	62	> 192	75th percentile
Yeast	60	> 65	75th percentile
<i>Aspergillus</i>	57	> 65	75th percentile
Dark unknown ^b	56	> 80	75th percentile
<i>Basidiomycetes</i>	41	> 64	80th percentile
<i>Acrodontium</i>	18	> 0	Detectable
<i>Alternaria</i>	16	> 0	Detectable

^aNon-sporulating fungi with colorless or light colored hyphae.

^bNon-sporulating fungi with dark hyphae.

(referent); and the season of visit, divided into spring (March–May), summer (June–August), fall (September–November), winter (December–February, the referent).

Home characteristics, observed at the time of the walk-through assessment, were examined as both independent predictors of wheeze and as potential confounders of fungi–wheeze associations. These characteristics included, visible mold, visible water (i.e., standing water, water damage, plumbing leaks), a moldy/musty/damp smell on entry into any room, the presence of dogs, cats, cockroaches, the use of a humidifier, home ownership, and the presence of throw rugs or wall to wall carpeting in the main living area. Each of these variables was dichotomous (yes/no) with “no” as the referent category. A mold severity index was also constructed by adding the number of occurrences of visible mold, water/dampness or musty odor within the home.

Statistical Analyses

The analyses were undertaken in several steps; the initial descriptive phase included the calculation of frequencies, means and SD. Pearson's χ^2 analysis was used for categorical assessments and Student's *t*-test or One-way ANOVA to compare means across two or more groups. In the analytic phase, both unadjusted and multivariable logistic regression modeling were employed to evaluate the associations between viable airborne fungi, bacteria and the occurrence of one or more episodes of wheeze during the first year of life. Odds ratios (OR) and 95% confidence intervals (CI) were calculated in all models. Effect modification (interaction) was assessed using backward elimination from the full model; a *P* value of ≤ 0.05 for the Likelihood ratio statistic was indicative of significant interaction. Potential confounders were determined based on the literature and on a $> 10\%$ change in the β -coefficient. Analyses were conducted using Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA).

Owing to the large number of potential independent variables, confounders, and covariables, preliminary χ^2 analyses were undertaken to characterize possible associations between: individual demographics; the demographics and home characteristics; the demographics and risk factors for infant wheezing observed in the literature (e.g., smoking, endotoxin levels); and, home characteristics, season of visit and fungal levels. Unadjusted logistic models were then constructed to assess the associations of each of the potential independent variables with wheeze in the first year of life. Demographics, infant and maternal characteristics, home characteristics (including pets and roaches), moving during the study, season of mold collection, total fungal levels, frequently identified taxa of fungi and reported risk factors were evaluated in this manner. The demographic, infant and home variables showing associations with wheeze were then evaluated as potential confounders in models in which one fungal taxon was the “primary exposure” variable. Based on preliminary findings, the presence of carpeting showed a strong inverse association with wheeze and was evaluated as an effect modifier and potential confounder in these models as well. Owing to the small sample size ($n = 103$), season of visit, risk factors for infant wheezing and only those variables demonstrating confounding of the primary exposure were retained in each of the final models.

Home characteristics such as visible mold and dampness also were evaluated in separate models as surrogates of the specific fungal taxa exposures. The modeling strategy employed was similar to that outlined for the measurable fungi in the previous paragraph.

Results

The AUDIT cohort is 45% male, 46% African American and 49% white, with 13% also reporting Hispanic ethnicity.

At study start, insurance coverage was 82% Medicaid, with the remainder listed as private. The mean (SD) maternal age at the time of pregnancy was 25.4 (5.8) years, with a range of 14–41 years. The majority (74%) of mothers were unmarried at delivery. Forty-six percent of mothers had less than a high school education (10% of total group were < 19 years of age), 28% were high school graduates and 26% reported some post high school education. In addition, 50% of mothers smoked during pregnancy, whereas 50% reported ever breast-feeding their child. A total of 39 infants (38%) experienced one or more episodes of wheezing during the first year of life.

As shown in Table 2, Medicaid insurance decreased maternal education and summer season of birth were associated with significantly increased risks of wheeze in the first year of life. Elevated odds ratios also were observed for male sex ($P=0.063$), non-white race, Hispanic ethnicity and being unmarried at the time of delivery; however, the confidence intervals included the null value. Breast-feeding showed an inverse association with wheeze ($P=0.059$), whereas maternal age demonstrated a positive non-significant association with wheeze; the OR (95% CI) was 1.04 (0.97–1.12) for each year increase in maternal age. Maternal smoking during pregnancy as well as smoking in the home during the first year of life was observed to have weak positive, but non-significant associations with wheeze in our study.

Nearly 90% of AUDIT participants lived in homes or apartments with wood frame construction. Housing of this type is typically a single family dwelling or a multi-flat house. Home ownership was reported by 15% of the participants, with 58% renting all or part of a house and the remainder renting apartments in an apartment complex or building. Even though remaining within the geographic area was required for study participation, over the course of the study, 62% of families moved from one home to another.

The mean age (SD) of infants at the time of home environmental sampling was 3.3 (1.6) months, with a median of 2.8 months. The baby's age at the home visit was not a significant determinant of wheeze (OR of 1.12 (95% CI of 0.88–1.44)). Environmental sampling occurred during all four seasons, with the risk of wheeze in the first year of life significantly higher among cohort members with a fall mold collection visit (Table 3).

Measurable levels of fungi were present in all homes (Table 1); the most frequently observed group was hyaline unknowns in 86% of homes followed by bacteria and *Penicillium*. *Acronotium*, a rarely observed genus was found in 18% of homes and *Alternaria*, in 16%. Fungal taxa found in fewer than 15% of homes, and not evaluated in this analysis, included *Zygomycetes*, *Actinomycetes*, *Aureobasidium*, *Acremonium* and *Trametes* species. Overall approximately 170 taxa (genus or species) were identified in the entire group of study homes.

Table 2. Demographic, infant and maternal characteristics and the risk of wheeze in the first year of life.

Variable	Any wheeze		OR ^a	(95% CI)
	<i>n</i> = 64 No (%)	<i>n</i> = 39 Yes (%)		
<i>Season of birth</i>				
Winter	31	15	1.0	Referent
Spring	28	23	1.67	(0.50–6.61)
Summer	22	49	4.52	(1.44–14.20)
Fall	19	13	1.40	(0.35–5.55)
<i>Race</i>				
White	53	42	1.0	Referent
Black/other	47	58	1.56	(0.69–3.50)
<i>Ethnicity</i>				
Non-Hispanic	89	84	1.0	Referent
Hispanic	11	16	1.53	(0.47–4.94)
<i>Sex of infant</i>				
Female	63	44	1.0	Referent
Male	37	56	2.16	(0.96–4.85)
<i>Mother's age at delivery, years</i>				
< 20	17	13	1.0	Referent
20–29	66	59	1.21	(0.37–3.89)
30 or more	17	28	2.20	(0.57–8.47)
<i>Mother's education</i>				
≤ High School	66	87	3.47	(1.18–10.19)
> High School	34	13	1.0	Referent
<i>Insurance</i>				
Private	27	5	1.0	Referent
Medicaid	73	95	6.69	(1.45–30.82)
<i>Mother's marital status</i>				
Married	30	20	1.0	Referent
Not married	70	80	1.64	(0.64–4.21)
<i>Ever breast feeding</i>				
No	42	62	1.0	Referent
Yes	58	38	0.46	(0.20–1.03)
<i>Maternal smoking, pregnancy</i>				
No	53	44	1.0	Referent
Yes	47	56	1.47	(0.66–3.27)
<i>Any smoker in household</i>				
No	36	26	1.0	Referent
Yes	64	74	1.63	(0.67–3.93)
<i>Day care/non-relative care</i>				
No	59	72	1.0	Referent
Yes	41	28	0.57	(0.24–1.35)

^aUnadjusted odds ratios (OR) and 95% Confidence Intervals (CI).

Descriptive measures of dampness and mold were also evaluated, with an overall median number of observations per home of two (mold severity scale), and with a range of

Table 3. Season of visit, home characteristics, pets/pests and the risk of wheeze in the first year of life.

Variable	Any wheeze		OR ^a	(95% CI)
	<i>n</i> = 64 No (%)	<i>n</i> = 39 Yes (%)		
<i>Season of mold collection</i>				
Winter	36	28	1.0	Referent
Spring	34	23	0.86	(0.30–2.46)
Summer	22	26	1.49	(0.51–4.42)
Fall	8	23	3.76	(1.02–13.92)
<i>Family move during study</i>				
No	39	36	1.0	Referent
Yes	61	64	1.15	(0.50–2.61)
<i>Visible mold</i>				
No	75	77	1.0	Referent
Yes	25	23	0.90	(0.35–2.29)
<i>Visible dampness/water</i>				
No	31	26	1.0	Referent
Yes	69	74	1.32	(0.54–3.22)
<i>Moldy/musty/damp odor</i>				
No	66	59	1.0	Referent
Yes	34	41	1.32	(0.58–3.02)
<i>Mold Severity Index^b</i>				
None	28	26	1.0	Referent
1–2	36	33	1.02	(0.36–2.85)
3 or more (above median)	36	41	1.25	(0.46–3.41)
<i>Use of humidifier</i>				
No	83	78	1.0	Referent
Yes	17	22	1.30	(0.47–3.61)
<i>Living room carpet/rugs</i>				
No	27	49	1.0	Referent
Yes	73	51	0.38	(0.16–0.88)
<i>Pet cat</i>				
No	75	80	1.0	Referent
Yes	25	20	0.77	(0.30–2.03)
<i>Pet dog</i>				
No	73	64	1.0	Referent
Yes	27	36	1.55	(0.66–3.65)
<i>Cockroaches</i>				
No	81	69	1.0	Referent
Yes	19	31	1.93	(0.76–4.86)
<i>Endotoxin (EU/mg dust)</i>				
< 100	73	51	1.0	Referent
> 100	27	49	2.62	(1.12–6.13)

^aUnadjusted odds ratios (OR) and 95% confidence intervals (CI).^bTotal number of observations of visible mold, dampness or water in the home.

0 to 13. Although dampness or water stains were observed by the environmental sampling team in 71% of residences, these characteristics were not associated with wheeze (Table 3). Other frequently observed indicators of mold within the homes included a moldy, musty or damp odor, detected in 37% of homes and visible mold, present in about 25% of homes. Neither of these home characteristics was associated with an increased risk of diagnosed wheeze among study infants. The addition of covariables and potential confounders to individual models assessing visible mold, visible water/dampness, or musty/moldy odors as independent predictors of wheeze resulted in similar odds ratios and 95% confidence intervals to those found in the unadjusted models and were consistent with no effect (multiple logistic regression results not shown). Among the assessed home characteristics, the presence of living room carpets or throw rugs showed a significant inverse association with wheeze.

The mean (SD) endotoxin level in homes was 109.7 (112.8) EU/mg dust, whereas the geometric mean was 76.1; home values ranged from 8 to 659 EU/mg dust. Elevated endotoxin levels were associated with a significant increase in wheeze in the first year of life; the unadjusted OR (95% CI) for values ≥ 100 EU/mg dust was 2.62 (1.12–6.13) (Table 3). The unadjusted continuous endotoxin odds ratio (95% CI) for each 20 EU/mg dust increase was 1.13 (1.04–1.22).

The correlation between mean indoor and outdoor levels of fungi and bacteria observed in at least 15% of homes are presented in Table 4. Although indoor and outdoor levels were correlated for several of the genera and for total fungi, outdoor genus-specific levels were not associated with wheeze in unadjusted logistic regression modeling, or in models with their respective indoor counterparts (data not shown). The lack of correlation between indoor and outdoor levels of *Aspergillus* and *Penicillium* supports the existence of indoor sources independent of outdoor levels for these two fungal taxa.

Further support for the view that indoor levels of *Penicillium* and *Aspergillus* are not solely dependent on outdoor levels appears in Figure 1; several of the genus-specific taxa were observed to fluctuate over the year with detectable levels more prevalent during the summer and fall seasons and non-detectable levels more prevalent in spring and winter for *Cladosporium*, *Alternaria* and *Acrodictum*. There was less variation in the percentage of non-detected fungi over the seasons for *Penicillium*, *Aspergillus* and *Basidiomycetes* species. These observations also are reflected in the mean indoor/mean outdoor (I/O) ratios for the taxa; *Aspergillus* and *Penicillium* had I/O ratios of 4.45 and 3.41, indicating higher average indoor than outdoor concentrations. *Basidiomycetes*, *Cladosporium*, *Alternaria* and *Acrodictum* species had I/O ratios of 0.90 or less and were consistent with greater concentrations of fungi outdoors than inside the residence. Overall, among the frequently observed taxa in this study, only *Penicillium* showed a statistically

significant positive association with visible mold ($P=0.044$), and weaker positive non-significant relationships with visible water ($P=0.069$) and reported musty/moldy odors ($P=0.095$).

Results from the logistic regression analyses of airborne fungi and bacteria with wheeze appear in Table 5. Among the genus-specific fungi, significant increases in wheeze were noted in the unadjusted analyses for exposure to “high” levels of *Aspergillus* ($P=0.04$) and *Penicillium* ($P=0.001$). “High” levels of *Cladosporium* also were positively associated with wheeze ($P=0.054$) as were detectable levels of *Acrodontium* ($P=0.051$). Exposure to measurable indoor levels of *Alternaria*, *Basidiomycetes*, hyaline unknowns, dark

unknowns, bacteria and yeast were not significantly associated with wheeze in unadjusted analyses. Among the total fungi, the odds ratios for the second through fourth quartiles were elevated compared to the referent in the unadjusted model, however, the confidence intervals included the null value.

Multiple logistic regression modeling (Table 5), undertaken to further assess the associations between airborne fungi and wheeze following adjustment for confounders and potential risk factors, indicated that exposure to “high” *Penicillium* levels remained a significant predictor of wheeze during the first year of life after controlling for season of visit, maternal smoking during pregnancy, any smoking in the home during the first year of life, endotoxin levels, day care attendance (surrogate for respiratory infections) and several demographic factors. The presence of visible mold, dampness/water or musty/moldy odor in the residence did not confound the measurable *Penicillium*–wheeze associations; consequently, none of these variables was included in the final model.

Owing to the correlation among indoor levels of several of the fungi and *Penicillium* (Pearson’s correlation coefficients ranged from 0.12 to 0.34), *Penicillium* levels were also evaluated in a logistic model with other genera. “High” levels of *Penicillium* remained a significant predictor of wheeze in a model which included season of visit, *Aspergillus*, *Cladosporium*, *Acrodontium*, maternal smoking during pregnancy, any smoking in the home, day care attendance and endotoxin levels; odds ratios and 95% CI were 1.33 (0.38–4.68) and 5.46 (1.15–25.89)

Table 4. Correlation of mean indoor and outdoor fungal levels (CFU/m³) ($n=103$).

	Correlation ^a	P-value
Total fungi	0.637	0.000
<i>Aspergillus</i>	0.005	0.959
<i>Penicillium</i>	0.107	0.281
<i>Cladosporium</i>	0.814	0.000
<i>Acrodontium</i>	0.555	0.000
<i>Alternaria</i>	0.599	0.000
<i>Basidiomycetes</i>	0.374	0.000
Hyaline unknown	0.698	0.000
Bacteria	0.168	0.089
Yeast	0.412	0.000
Dark unknown	0.742	0.000

^aPearson’s correlation coefficient.

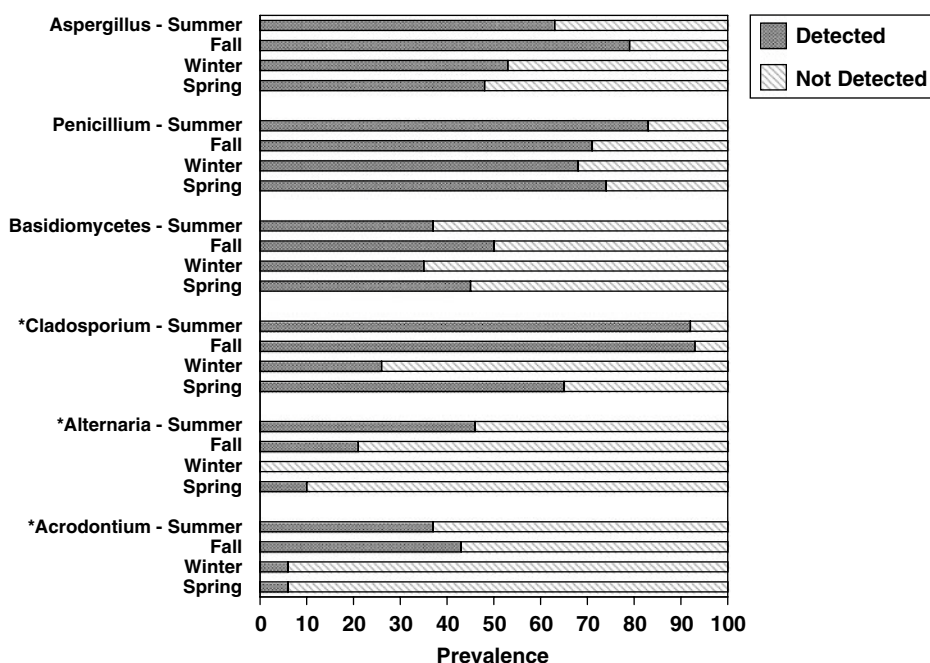


Figure 1. Prevalence of indoor fungi by season of home visit. * $P<0.05$ in Pearson’s χ^2 test.

Table 5. Mean^a indoor airborne fungi and the risk of wheeze in the first year of life.

Fungi (CFU/m ³)	Any wheeze		Unadjusted		Adjusted ^c	
	n = 64	n = 39	OR ^b	(95% CI)	OR	(95% CI)
	No (%)	Yes (%)				
Total fungi						
1st Q ^d (56–268)	29	18	1.0	Referent	1.0	Referent
2nd Q (269–571)	21	33	2.57	(0.80–8.23)	3.64	(0.67–19.65)
3rd Q (572–1214)	25	23	1.45	(0.44–4.78)	0.77	(0.15–3.93)
4th Q (1215–4770)	25	26	1.61	(0.50–5.22)	0.96	(0.19–4.84)
<i>Aspergillus</i>						
Not detected	52	28	1.0	Referent	1.0	Referent
Low (16–64)	28	39	2.50	(0.95–6.58)	1.27	(0.41–3.98)
High (65–2604)	20	33	3.00	(1.07–8.39)	1.58	(0.43–5.79)
<i>Penicillium</i>						
Not detected	33	15	1.0	Referent	1.0	Referent
Low (16–119)	55	39	1.50	(0.50–4.46)	1.80	(0.50–6.55)
High (120–1270)	12	46	7.88	(2.30–26.99)	6.18	(1.34–28.46)
<i>Cladosporium</i>						
Not detected	44	28	1.0	Referent	1.0	Referent
Low (16–191)	36	36	1.55	(0.59–4.06)	2.11	(0.51–8.74)
High (192–1715)	20	36	2.74	(0.98–7.66)	2.28	(0.41–12.67)
<i>Acrodontium</i>						
Not detected	88	72	1.0	Referent	1.0	Referent
Detected (16–478)	12	28	2.75	(0.99–7.61)	1.72	(0.49–6.03)
<i>Alternaria</i>						
Not detected	84	82	1.0	Referent	1.0	Referent
Detected (16–191)	16	18	1.18	(0.41–3.41)	0.96	(0.27–3.45)
<i>Basidiomycetes</i>						
Not detected	58	62	1.0	Referent	1.0	Referent
Low (16–63)	20	20	0.95	(0.34–2.63)	0.76	(0.24–2.40)
High (64–2191)	22	18	0.77	(0.27–2.19)	0.77	(0.24–2.49)
<i>Hyaline unknown</i>						
1st Q (ND ^e –33)	25	26	1.0	Referent	1.0	Referent
2nd Q (34–142)	28	18	0.62	(0.19–2.02)	0.44	(0.11–1.68)
3rd Q (143–381)	22	30	1.37	(0.46–4.14)	0.64	(0.18–2.32)
4th Q (382–2159)	25	26	1.00	(0.33–3.06)	0.71	(0.20–2.52)
<i>Bacteria</i>						
Not detected	23	23	1.0	Referent	1.0	Referent
Low (16–127)	46	59	1.32	(0.49–3.56)	1.06	(0.35–3.21)
High (128–4000)	31	18	0.58	(0.18–1.92)	0.60	(0.16–2.20)
<i>Yeast</i>						
Not detected	39	41	1.0	Referent	1.0	Referent
Low (16–64)	36	33	0.88	(0.35–2.23)	0.61	(0.19–1.96)
High (65–413)	25	26	0.98	(0.36–2.68)	0.76	(0.23–2.57)
<i>Dark unknown</i>						
Not detected	49	36	1.0	Referent	1.0	Referent
Low (16–79)	28	36	1.72	(0.67–4.42)	1.37	(0.44–4.21)
High (80–604)	23	28	1.62	(0.60–4.42)	1.01	(0.27–3.74)

^aMean of three values collected at home visit over 24 h.^bOdds ratios (OR) and 95% confidence intervals (CI).^cAll models adjusted for season of visit, maternal smoking during pregnancy, any smoker in home, day care center or non-relative care, endotoxin levels. Total fungi also adjusted for insurance, mother's education, baby's race and any living room carpet. *Aspergillus* also adjusted for insurance, mother's education and baby's gender. *Penicillium* also adjusted for insurance, baby's gender, mother's age and baby's age at mold collection visit. *Cladosporium* also adjusted for mother's education, baby's gender, mother's age and baby's age at mold collection visit.^dQ = Quartile.^eND = not detected.

for low and high *Penicillium*, respectively compared to homes with non-detectable levels.

The addition of potential confounders and known risk factors to individual models evaluating *Cladosporium*, *Aspergillus* and *Acrodonium* exposure weakened the unadjusted associations between each genus and wheeze and were consistent with no effect. Following adjustment for potential confounders, including carpeting and potential risk factors, the total fungi–wheeze odds ratios shifted slightly but remained consistent with the null effect as well (Table 5).

Examination of possible associations between *Basidiomycetes*, bacteria, yeast, hyaline unknowns, dark unknowns and wheeze in both unadjusted and adjusted logistic regression models with control for covariables, yielded null findings. Covariables included season of visit, maternal smoking during pregnancy, environmental tobacco smoke in the first year of life, endotoxin levels, day care attendance, home characteristics and demographic factors. Both the unadjusted models and those with adjustment for season of visit and known risk factors for infant wheeze are shown in Table 5.

Discussion

High indoor levels of *Penicillium* were significantly associated with one or more episodes of wheeze during the first year of life. These findings were independent of season of home sampling, the baby's age at the time of the visit, gender, the mother's age, insurance type and several risk factors for infant wheeze including: day care attendance (Ball et al., 2000; Stark et al., 2003), endotoxin levels (Park et al., 2001), maternal smoking during pregnancy (Gold et al., 1999; Lannero et al., 2006) and exposure to environmental tobacco smoke (Strachan and Cook, 1997; Stein et al., 1999). Although "high" levels of *Aspergillus*, *Cladosporium*, and *Acrodonium* demonstrated positive associations with wheeze in unadjusted analyses (each P value was ≤ 0.05), the odds ratios decreased following the addition of season of visit and other covariables. In each of these multiple logistic regression models, the confidence interval included the null value and was wide which reflected the small sample size and lack of precision in the effect estimates.

Our findings of a positive association between indoor *Penicillium* levels and wheeze during the first year of life are consistent with results from two cohort studies from the northeastern part of United States. In a Connecticut-based study, high levels (>1000 CFU/m³) of *Penicillium* significantly increased the risk of both wheeze and persistent cough in the first year of life (Gent et al., 2002). The risk of wheeze was stronger among children whose mothers' had a diagnosis of asthma (Belanger et al., 2003). Stark et al. (2003) reported a significant association between indoor airborne *Penicillium* (levels above 189 CFU/m³) and lower respiratory tract illness

without wheeze among infants in a Boston birth cohort study. The same group also found a marginally significant increased risk of *Penicillium* and lower respiratory tract illness with wheeze; the relative risk (RR (95% CI)) was 1.56 (0.92–2.65). In our cohort, all mothers had diagnosed asthma, while in the Boston study, eligibility criteria included at least one parent with a history of asthma or allergy as well. Results from the Connecticut-based study suggest that children with a maternal history of asthma may be more susceptible to the effects of fungal exposures (Douwes and Pearce, 2003).

Stark et al. (2003) reported significant associations between dust-borne *Zygomycetes* and *Alternaria* and lower respiratory tract illness with wheeze in the first year of life. Airborne *Zygomycetes* was recovered in too few of our study homes for analysis whereas detectable *Alternaria* levels were not associated with wheeze in the AUDIT cohort.

Numerous studies (primarily cross-sectional in design) involving older children and adults have reported at least one positive association between total airborne fungal or bacterial counts, or specific fungal taxa including *Penicillium*, *Cladosporium*, *Aspergillus* or *Alternaria* species, and respiratory symptoms including cough, wheeze and asthma (Platt et al., 1989; Strachan et al., 1990; Bjornsson et al., 1995; Li and Hsu, 1997; Garrett et al., 1998; Dharmage et al., 2001; Downs et al., 2001). Null findings in all age groups also have been reported for both airborne (Su et al., 2001) and dust-borne fungi (Wickman et al., 1992; Verhoeff et al., 1994). Some studies included participants with known diagnoses of asthma or allergies which may have been exacerbated by exposure to fungi (Bjornsson et al., 1995; Li and Hsu, 1997; Garrett et al., 1998; Dharmage et al., 2001).

Mold levels tend to be highest in the summer and fall throughout the United States (Shelton et al., 2002), and were observed in this project as well. Children born in the summer and those with fall mold collection dates, an overlapping group in our study, were at an increased risk of wheeze in unadjusted logistic regression models. These observations could represent exposure to relatively high indoor fungal levels at a young age or exposure to respiratory syncytial virus or rhinovirus at an age when small airway size can be a factor in the development of wheeze during viral infection (Heymann et al., 2004; Sears and Johnston, 2007). Nonetheless, "high levels" of *Penicillium* remained a significant predictor of wheeze even after accounting for the effects of season of mold collection, the age of the baby at the sample collection date and day care attendance, a surrogate measure of respiratory infection during infancy and early childhood (Ball et al., 2000; Stark et al., 2003).

To our knowledge, the association between detectable levels of *Acrodonium* and wheeze has not been reported earlier. *Acrodonium myxomyceticola* made up 83% of the *Acrodonium* species identified in indoor and outdoor air samples in study homes; the subsampling procedure, which

allows for slower growing fungi, likely facilitated recovery of this rarely reported genera (Fernando et al., 2005; Catranis et al., 2006). Other infrequently observed fungi recovered in AUDIT homes using this new procedure included *Acremonium roseolum*, *Gnonmonia* species, *Myxotrichum deflexum*, *Aphanocladium album*, *Phialophora botulisporea* and *Tetracoccusporium paxianum* (Fernando et al., 2005). Use of this new subsampling procedure would be recommended for studies in which recovery of rare fungi is essential; the procedure is complex and labor intensive necessitating consideration of these factors during the study design phase.

A home inspection during air sampling showed the majority of participant homes had visible dampness, water, mold, or a musty odor. In contrast to other birth cohort studies (Belanger et al., 2003, 2006; Cho et al., 2006), home characteristics, such as dampness or visible mold, were not associated with wheeze in AUDIT infants. Moreover, *Penicillium* was the only frequently occurring genus in our study showing a significant association with visible mold and somewhat weaker associations with visible water and musty/moldy odor. *Penicillium* also exhibited higher indoor than outdoor levels in our study, an observation reported by others throughout the United States (Shelton et al., 2002; Chew et al., 2003; O'Connor et al., 2004). The ubiquitous nature of *Penicillium* sp. in the indoor environment may provide greater opportunities for exposure among residents year-round and account for the consistency of effects across studies.

AUDIT is one of the several studies with both measured fungal counts and observed dampness and mold. Results have shown null, weak, and significant positive associations among the two exposure types as well as inconsistencies across studies with respect to the taxa showing associations with home characteristics (Waegemaekers et al., 1989; Strachan et al., 1990; Wickman et al., 1992; Li and Hsu, 1997; Garrett et al., 1998; Ren et al., 2001; Chew et al., 2003; O'Connor et al., 2004). These inconsistencies could be secondary to geographic location, season of sampling, as well as housing types and construction materials. Methodological differences in sampling, plating and identifying fungi as well as differences among respondent and observer protocols for evaluating home characteristics also could contribute to inconsistencies across studies. Fungal growth may occur under carpets and behind the wall board and appliances, leading to under-reporting of these characteristics. Common fungi found in water-affected buildings include *Penicillium* and *Aspergillus* species as well as *Stachybotrys chartarum*. Typical outdoor fungal genera (e.g., *Cladosporium*, *Alternaria* and *Basidiomycetes*) are often tracked in or blown indoors during normal activities (Dillon et al., 1999).

Carpeting was found to have a significant protective effect in our cohort (Table 3). Although seemingly counter-intuitive, carpeting appears to be associated with socio-

economic status in our study population; home owners were more likely to have private health insurance and also were more likely to have carpeting. A similar observation was made in a comparison of suburban and inner city homes of children with asthma (Simons et al., 2007). In a European birth cohort, carpeting in the child's bedroom was uncommon and not related to wheezing during the first year (Hagendorens et al., 2005).

Elevated house dust endotoxin from the main living area was also associated with an increase in infant wheeze in the AUDIT birth cohort. These findings are consistent with those reported by Parks et al., 2001 although their geometric mean was somewhat higher than that observed in our study, 100 versus 76 EU/mg dust.

The relationship between early wheezing and the development of asthma later in childhood remains poorly understood. Although the majority of wheezing episodes among infants appear to be transient, in a subgroup of infants, including those whose mothers have asthma, wheezing episodes may reflect a predisposition to asthma (Martinez et al., 1995). It is interesting to note whether exposure to measurable fungi during infancy plays a causal role in the development of allergies and asthma later in childhood. Evidence suggests that the development of asthma occurs primarily during early childhood and involves both genetic and environmental factors including biologics (e.g., allergens, viruses, bacterial products). The timing and "dose" of the environmental exposures and the interaction with genes and other susceptibility factors (e.g., breast-feeding, lifestyle) during specific developmental periods appear critical to the induction of asthma (Martinez, 2003; Yeatts et al., 2006; Gilmour et al., 2006; Zeldin et al., 2006; Selgrade et al., 2006). It is likely that fungal allergens eliciting immunological and airway changes during this critical developmental period are dependent in part, on the geographic area and climate (Martinez, 2002), perhaps accounting for the variety of fungal taxa-respiratory symptom associations which have been observed.

Although follow-up data are not currently available for the AUDIT population, additional longitudinal results from the New England birth cohort studies do provide support for a causal role of early fungal exposure in the development of both allergies and asthma. A significant increase in asthma diagnoses by 6 years of age was observed among Connecticut cohort participants exposed to measurable airborne *Penicillium* as infants (Belanger et al., 2006). Exposure to elevated levels of dust-borne *Aureobasidium*, *Aspergillus* and yeasts during infancy was associated with significant increased risks of allergic rhinitis by 5 years of age among Boston birth cohort members (Stark et al., 2005).

Limitations of the AUDIT study include a small sample size which impacted the precision of the point estimates and our ability to see relatively small increases or decreases in risk of wheeze among cohort members. Observed odds ratios for

several variables (e.g., gender, breast-feeding, smoking) in the AUDIT study were consistent with those in other studies but remained non-significant (Martinez et al., 1995; Gold et al., 1999); the presence of cockroaches was also associated with a non-significant elevation in the odds ratio. Eligibility requirements limited the pool of potential participants to women with a diagnosis of asthma in their third trimester of pregnancy who resided within Syracuse, NY, a small city with a population of 147,306 (US Census for, 2000). The number of births to Syracuse residents during 2001 and 2002 total is about 4400 (New York State Department of Health Vital Statistics, City of Syracuse). The identification of 370 pregnant asthmatics (out of about 3000 births) over the 16-month recruitment period would result in an asthma prevalence of approximately 12%; this is the reported prevalence for asthma in US adults, and in the New York State 2002 Behavioral Risk Surveillance Survey (CDC, 2002). Although it was known in advance that the sample would be small, characterization of the home environment and health among inner-city infants at high risk for asthma in a community of this size has been infrequently studied and remains a public health concern.

Despite assurances to the contrary, 62% of the study sample moved at least once during the study; consequently, the environmental sampling may have occurred at one home and the wheezing episodes at another. Bioaerosol collection occurred relatively early in the study period (median age of infants was 2.8 months) and the majority of participants remained within the city of Syracuse where housing stock tends to be similar in age and quality. Moving was not associated with wheeze in our study (Table 2), although it was significantly associated with both lower maternal educational achievement and Medicaid insurance (data not shown), two demographic factors with significant positive relationships to wheeze (Table 1). Among the assessed fungal taxa, only *Penicillium* showed a significant positive association with having moved. Higher levels of *Penicillium* also were associated with both Medicaid insurance and lower maternal education, suggesting a complex relationship between socio-economic factors, moving and measurable *Penicillium* levels. Nonetheless, when the variable “moved” was added to the multivariable *Penicillium* model which included insurance and other covariables (Table 5), it was not found to be an effect modifier or a confounder (data not shown) of the *Penicillium* point estimates.

As in most studies conducted to date assessing indoor air quality and respiratory symptoms, indoor airborne fungal sampling in the AUDIT study was of short duration, 3 or 6 min; consequently, the concentrations do not represent long-term exposure levels, but rather a snapshot of the indoor environment at the time of sampling. Strengths of our bioaerosol sampling protocol included: three replicate samples in a 24-h period, outdoor sample collection at each residence and sampling in all four seasons. Airborne

sampling of viable fungi also allowed for growth and identification of fungal isolates to the genus and species levels.

Conclusions

Total fungal levels and home characteristics such as dampness/visible water and visible mold were not associated with wheeze among AUDIT study members; however, *Penicillium* demonstrated a significant positive association with visible mold in the residence. In multivariable logistic regression modeling, elevated indoor airborne *Penicillium* remained a significant predictor of wheeze in the first year of life among infants at risk for the development of asthma even after controlling for season of visit, demographic characteristics and several known risk factors for infant wheeze.

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