11

Statistical Considerations for Bioaerosol Health-Risk Exposure Analysis

M.D. Larrañaga[1], E. Karunasena[2], H.W. Holder[3], E.D. Althouse[4] and D.C. Straus[5]
[1]*Oklahoma State University*
[2]*Texas Tech University*
[3]*SWK, LLC*
[4]*Air Intellect, LLC*
[5]*Texas Tech University Health Sciences Center*
USA

1. Introduction

Air and surface sampling was conducted to confirm the types of microbiological contamination within a hospital facility in the southern United States, identify indicators of indoor microbiological contamination, and profile the aero-biological makeup of the inside air (ISA) for comparisons to outside air (OSA) and reference concentrations, where applicable. The investigation strategy recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) was utilized to assess indoor environmental quality and conditions found within the hospital facility. The ACGIH methodology is guided by the text Bioaerosols: Assessment and Control. (Macher 1999) Biological contamination within a hospital environment is of great concern as bacteria and fungi are important causes of nosocomial infection (NI). It is estimated that the overall hospital-acquired infection rate in Europe and North America is between 5 and 10%. (Kalliokoski 2003) A substantial number of bacteria and fungi are capable of spreading via the airborne route in hospitals, and airborne transmission accounts for approximately 10% of all NI. (Eickhoff 1994; Kalliokoski 2003) Contaminated Heating, Ventilating, and Air Conditioning (HVAC) systems and infiltration of unfiltered outside air have been implicated in airborne outbreaks of NI via infective aerosols, dust, and contaminated filters. (Lentino, Rosenkranz et al. 1982; Rhame 1991; Eickhoff 1994) Certain underlying diseases, procedures, hospital services, and categories of age, sex, race, and urgency of admission have been shown to be significant risk factors for nosocomial infection. (Freeman and McGowan 1978) The day-specific incidence of nosocomial infection rises from near zero on the first hospital day to maximal during the fourth through seventh weeks of hospital stay. (Freeman and McGowan 1981) Nosocomial infections can affect patients in any location within a hospital. (Boss and Day 2003)

The Centers for Disease Control (CDC) estimates that 2 million patients develop hospital-acquired infections annually and as many as 88,000 die as a result. (CDC 1992) Hospitals typically maintain a patient population with increased susceptibility to infection and factors

inherent to the healthcare environment contribute to the risk associated with acquiring an infection during a hospital admission. (Dulworth and Pyenson 2004) Environmental control and high efficiency filtration are critical to preventing person-to-person and environmentally related infections in hospitals. (Wenzel 1997; Boss and Day 2003) Current evidence indicates that excessive moisture indoors promotes microbial growth and is associated with an increased prevalence of symptoms due to irritation, allergy, and infection. It is widely accepted in various scientific communities that indoor microbiological contamination presents unacceptable conditions for the preservation of human health, and that removal and prevention of microbial contamination is necessary and prudent. (Pope, Patterson et al. 1993; Macher 1999; Agency 2001; ACOEM 2002; Fung and Hughson 2002; Redd 2002; CDC 2003; Fung and Hughson 2003)

The inherent variability of microbiological organisms in air presents a challenge for conducting air sampling that provides meaningful results for the evaluation of human exposure and health risk. In general, bioaerosol air sampling is highly variable and prone to error. Multiple and replicate samples over subsequent days are necessary to characterize exposure and multiple samples per sample location are required to evaluate human exposure in that particular location. An air and surface sampling plan was designed to address the inherent variability and error associated with air sampling and to evaluate the exposure and subsequent health risk to patients, visitors, and staff in a hospital facility. The objectives of this chapter are to highlight the necessity for multiple (replicate) air samples per sample location to conduct valid assessments of the airborne concentrations of bioaerosols.

2. Materials and methods

Sampling was conducted to provide a bioaerosol profile of the air within the spaces under the control of 9 separate HVAC systems over a two-year period. The spaces under the environmental control or each HVAC system are identified as air handling units (AHUs) 10, 11 (no final filters present), 13 (90% final filters), 15, 16, 17, 18, and 19 during the summer of 2005. In early 2006, a follow-up investigation was conducted within the spaces controlled by AHUs 17, 19, and 21. Except where indicated, AHU final filters had a filtration efficiency of 95%. Air and surface sampling data and analysis, observations, and the collective experience of a team experienced in moisture intrusion in hospital facilities along with input from professionals in medical microbiology, industrial hygiene, medicine, engineering, and public health were utilized to interpret data for hypothesis testing. The hypotheses were:

a. Hypothesis A: The 90-95% final filters control particulate matter generated by the AHUs and preventing contamination downstream of the filters. Note: AHU 11 does not have final filters and therefore this hypothesis does not apply to AHU 11.
b. Hypothesis B: The 90-95% final filters prevent microbial contamination downstream of the filters. Note: AHU 11 does not have final filters and therefore this hypothesis does not apply to AHU 11.
c. Hypothesis D: Staff is being exposed to potentially harmful concentrations of biological contaminants.
d. Hypothesis E: Patients are being exposed to harmful quantities of biological contaminants.

Air sampling was conducted to establish mean airborne concentrations of fungal and biological aerosols indoors and outdoors to characterize the fungal and bacterial

aerobiological profiles of the areas controlled by each AHU. Culturable air samples were analyzed at the species level as information on species is crucial for determining whether indicator organisms are present. Indicator species of fungi whose presence indicates excessive indoor moisture or a health hazard were evaluated. Indicator organisms identified via air sampling in the hospital were *Aspergillus versicolor, A. flavus*, *A. fumigatus*, *Fusarium* species, yeasts, and species of *Penicillium*. (Macher 1999) In addition, the American Industrial Hygiene Association (AIHA) has consistently recommended urgent risk management decisions be made when the confirmed presence of these indicator organisms are identified indoors These indicator organisms include those listed above in addition to *Stachybotrys chartarum*. The confirmed presence is defined as colonies in several samples, many colonies in any sample, or, where a single colony was found in a single sample, evidence of growth of these fungi on building materials by visual inspection or source sampling. (Macher 1999) As early as 1996, AIHA stated that urgent risk management decisions are required of the industrial hygienist in the following conditions: a) the confirmed presence of facultative pathogens (fungi capable of inducing pulmonary infections in humans) such as *A. versicolor and Fusarium moniliforme,* and b) The presence of fungi, such as *S. chartarum* and *F. moniliforme*, known to result in occupational diseases in part due to their potent toxins. AIHA recommended that these urgent risk management decisions be made promptly as opposed to weeks or months later. (Dillon, Heinsohn et al. 1996; Prezant, Weekes et al. 2008) Based on the literature and in consideration of the recommendations made elsewhere by governmental and nongovernmental entities and other professional societies, the 1996 and 2008 AIHA recommendations and the 1999 ACGIH recommendations (Macher 1999) continue to be appropriate risk management guidance for the industrial hygienist and indoor environmental professional.

The estimated cumulative sampling and analytical error for each air sample is defined as $E_C = (P^2 + T^2 + A^2 + O^2)^{1/2}$ where E_C is the cumulative error, P is the pump error (estimated at ± 5%), T is the time error (estimated at ± 2.5%), A is the analytical error (estimated to be ± 25%), and O is other error associated with calibration and technician variability (estimated to be ± 25%). (Macher 1999; Burton 2006) Solving for E_C, the cumulative sampling and analytical error for each sample is estimated to be approximately ± 36%. Therefore, data derived from individual samples should be viewed as qualitative. The inherent variability of the air concentrations of bioaerosols over time or within a space far outweighs any errors associated with measurement of airborne microbiological concentrations. Thus, the interpretation of a single sample is difficult without information on the variability of the concentrations of biological agents identified in the environment because the variability in the measurement is almost always large. (Macher 1999)

Duplicate, side-by-side air samples were taken at each location to address the error associated with individual samples. Duplicate side-by-side air sampling is considered adequate to define the mean and the random sampling error given the high temporal and spatial variability of bioaerosol concentrations in air. (Dillon, Heinsohn et al. 1996) Multiple samples from multiple random and non-random locations were taken on separate days to characterize exposures within the space under the environmental control of each air handling unit. (Macher 1999) Replicate samples within the space controlled by each air handling unit were taken to address sampling variability (Weber and Page 2001) and allow for the estimation of the sampling data's variances so that differences between two environments (e.g. ISA v. OSA concentrations) could be identified. (Macher 1999) A minimum of 6 replicate samples were taken at 10 indoor locations ($6 \leq n \leq 48$ samples per

location) and 2 outdoor locations ($12 \leq n \leq 72$ total outdoor reference samples per indoor location). An ANOVA was utilized to compare indoor and outdoor concentrations of biological agents using SAS Statistical Software. The level of significance was prescribed as $\alpha = 0.05$. A significant difference identified by the ANOVA indicates that the difference is unlikely to have occurred by chance and that there is statistical evidence that there is a difference. At the $\alpha = 0.05$ (5%) level of significance, the result could have occurred by chance one time in 20. Statistical techniques evaluate an observed difference in view of its precision to determine with what probability it might have arisen by chance (the level of significance). Values with a low probability of occurring by chance are called statistically significant and are considered to represent a real effect (e.g. difference between means). (Conover 1999; Montgomery 2001)

Ideally, human respiratory exposure is measured using air samples taken near the breathing zone, or within 12 inches of the mouth. This was not feasible considering the large size and weight of bioaerosol air sampling equipment. Most bioaerosol sampling is done to characterize ambient aerosols and the ambient conditions are utilized as quantitative estimates of bioaerosol exposure. Although the characterization of the ambient environment is not the ideal exposure sampling scheme, when low flow rate suction impactors (e.g. Andersen type) are utilized, the error introduced is small. (Pope, Patterson et al. 1993) Low flow rate suction impactors were utilized to take bioaerosol samples.

A comparison of total fungal or bacterial concentrations may be used as a preliminary indicator of a difference in two environments, but not as evidence of similarity or dissimilarity. Indoor/outdoor comparisons are used to document the presence or infer the absence of indoor, biologically derived contamination. These comparisons cannot be made unless the genera and species found indoors and outdoors have been identified. (Macher 1999) Air sampling mean total counts were utilized as a preliminary indicator of a difference in two environments (e.g. indoor vs. outdoor air concentrations) to determine the effectiveness of the filters in removing aerobiological particulates from the air stream. Air sampling indicators of indoor microbiological contamination were identified from sampling results where both the genera and species are identified (culturable fungal and bacterial samples incubated at 25°C and 37°C). Culturable air samples were taken for incubation at two separate temperatures (25°C and 37°C) to enhance the detection of both environmental and pathogenic microorganisms in the air. (Dillon, Heinsohn et al. 1996; Macher 1999)

Air sampling indicators of indoor contamination were compared to both surface sampling results and outdoor air sampling concentrations of like-organisms for interpretation. Note that monitoring for allergens can help characterize environments with respect to specific allergens (e.g., fungi and/or bacteria), and measurements can be semi-quantitative (e.g., "presence or absence" or "low, medium, or high"). (Pope, Patterson et al. 1993) Airborne bioaerosol sampling was conducted so that comparisons and interpretations could be made between ISA and OSA culturable and non-culturable bioaerosols, mean concentrations and variability of concentrations, and species could be compared. Each set or type of sampling results should be viewed in consideration with the other sampling results and not independently. For example, one should not consider spore trap (total and non-culturable fungal samples) alone, as spore trap sampling does not identify fungi at the species level and may mask important differences in species present between test and reference locations. Analysis of spore traps alone or total fungal or bacterial concentrations could lead to incorrect conclusions. The sampling interpretation considered all sampling results for the development of interpretations and conclusions.

The American Society for Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) defines critical care areas as the following functional spaces within a hospital: 1) Surgery and Critical Care, 2) Nursing, 3) Ancillary, 4) Diagnostic and Treatment, and 5) Sterilization and Supply. Critical care areas include but are not limited to intensive care units, coronary care units, angiography laboratories, cardiac catheterization laboratories, delivery rooms, operating rooms, recovery rooms, emergency departments, and other special care units where enhanced engineering controls are required for the protection of patients and staff. Although patients spend most of their time within a specific area, they may be exposed to bioaerosols in non-critical locations of a hospital where engineering controls are not as stringent. Non-critical functional areas are administration and service locations within the Hospital. (ASHRAE 2003) Table 1 lists the critical and non critical locations within the areas investigated.

AHU #	Area Served	Critical Area
10	Endoscopy, EKG, EGG, PFT, Pharmacy, Sterile Preparation, Recovery	Yes
11	1st floor PBX, Waiting Room, HR, Medical Records, Security	No
13	Patient Rooms	Yes
15	Intermediate Nursery, Ambulatory Surgical Center, Endoscopy, Operating Rooms, Sterile Supply, Pre- and Post- Op areas	Yes
16	Patient Rooms, Wound Therapy, Lactation Center	Yes
17	Emergency Room, Radiology and CT-Scan	Yes
18	Radiology, Ultrasound, Mammography, Laboratory, Laboratory Biohazard, Histology, Pathology	Yes
19	1st Floor, Administration Area, Labor and Delivery, Recovery, C-Section Room, Pre-Op Area, Intensive Care, Rehabilitation	Yes
21	Radiology, Purchasing, Shipping and Receiving, Plant Operations	Yes

Table 1. Classification of Hospital areas by critical or non-critical area.

3. Results

3.1 Total outside vs. total indoor concentrations (2005 data)

Figure 1 depicts the percent difference of bioaerosols from the OSA reference concentrations to the ISA concentrations for the space controlled by each AHU. The results show that the existing filters within the facility were removing bioaerosols from the air within the Hospital.

95% final filters were installed in the AHUs investigated, with the exception of AHUs 11 and 13. AHU 11 did not have final filters and AHU 13 had 90% final filters installed. Ninety-five percent final filters are rated to remove greater than 90% of particles between 1.0 and 10 micrometers in size and 85-95% of particles between 0.3 and 1.0 micrometers in size. Ninety percent final filters are rated to remove 90% of particulate matter between 1 and 10 micrometers in size and 75-85% of particles between 0.3 and 1.0 micrometers in size.

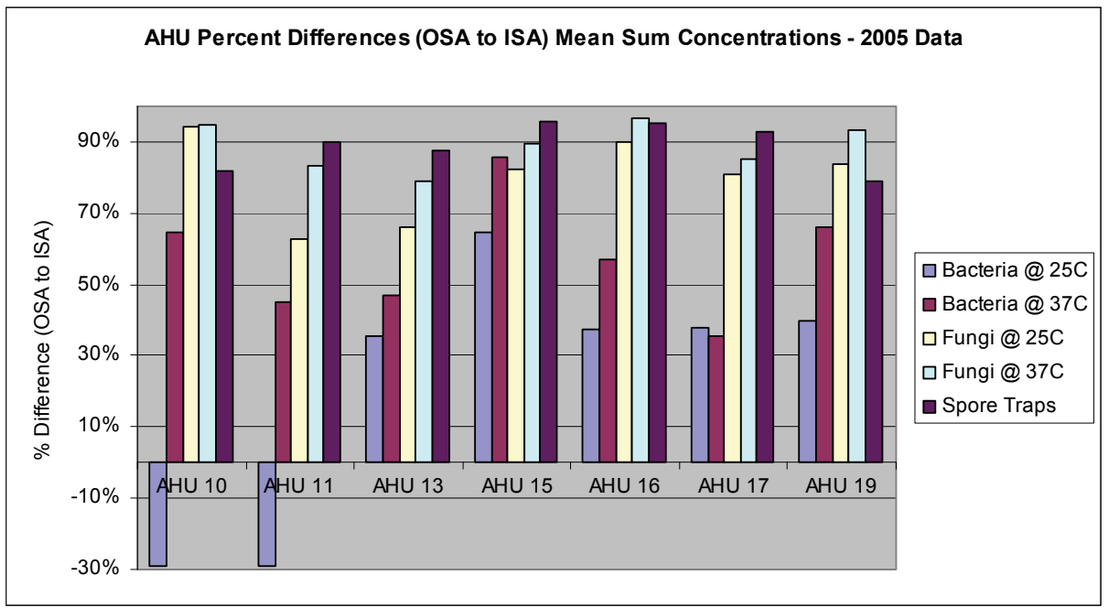


Fig. 1. AHU percent differences from outside air to inside air.
Note: A negative percent difference indicates that the indoor concentration was higher than outdoors.

(ASHRAE 1992; ASHRAE 1999) Therefore, with the exception of AHU 11, the percent differences for fungal particulates between OSA and ISA should approach 90% for organic (e.g. fungi and bacteria) particulate matter ranging from 1.0 to 10 micrometers in aerodynamic diameter and 78-85% of organic matter for particles ranging from 0.3-1.0 micrometers in aerodynamic diameter.

Non-culturable fungal air sampling via spore trap measures the airborne fungal particle concentrations in spores/m^3 of air. The minimum percent difference (reduction) of 79% (AHU 19) for total spore count (spore trap) results indicates that at least 79% of the non-culturable total spore concentrations from the outside air are being removed by the HVAC systems prior to entering the building. The minimum percent difference of all fungal air sampling results is 63% for the fungal samples incubated at 25°C in the space controlled by AHU 11. Excluding AHU 11 because it does not have final filters, the minimum percent difference of all fungal sampling results is 66% for AHU 13, which has 90% final filters. This indicates that at least 66% of the fungal bioaerosols are being removed by the final filters of the AHUs investigated, with the exception of AHU 11. The concentrations of total and culturable fungal bioaerosols within the spaces controlled by the AHUs with 95% final filters (all except AHUs 11 and 13) are at least 75% lower than outside air concentrations, indicating that the filters are performing and removing particulates from the air.

The bacterial concentrations (incubated at 25°C) within the space controlled by AHUs 10 and 11 were higher indoors than outdoors. Unlike fungi, bacteria have natural reservoirs indoors (including humans), and total bacterial concentrations are often higher indoors than outdoors. (Macher 1999) The bacterial organisms identified (incubated at 25°C) as indicators of an indoor source were *Micrococcus* species, *Micrococcus luteus, Staphylococcus capitus*, and *Staphylococcus hyicus,* which are human-shed bacteria. (Wilson 2005) Because these

organisms are human-shed and were not identified as indoor contaminants via surface sampling, it cannot be concluded that these higher concentrations of bacteria detected via air sampling were the result of building-related sources of bacterial contamination. (Macher 1999) Higher indoor concentrations of human-shed bacteria are an anticipated condition within a building. The bacteria *Bordetella bronchiseptica* was identified inside AHUs 10, 11, 13, 16, 18, 19 and was not identified indoors via air sampling. Viridans streptococci was identified inside AHU 18 and not identified in indoor air samples. *Staphylococcus aureus* was identified inside AHU 21 and not identified in indoor air samples. This is a strong indication that the filters may prevent the transmission of *Bordetella bronchiseptica*, Viridans streptococci, and *Staphylococcus aureus* through the filters and into the occupied space of the hospital.

With the exception of the bacterial (incubated at 25°C) air samples in AHUs 10 and 11, the percent differences for bacterial sample sets comparing OSA to ISA were at least 35%. This is an indication that the filters were removing bacteria from both the outside and re-circulated air of the Hospital.

3.2 Total outside vs. total indoor concentrations (2006 data)

The 2006 sampling data indicate that, in general, the total air concentrations are lower indoors than outdoors. However, indicators of indoor contamination were identified. The 2006 sampling strategy was to compare outdoor fungal and bacterial airborne concentrations with the concentrations identified 1) within each AHU before the filter, 2) within each AHU after the filter, and 3) within a room controlled by the AHU. AHUs 17, 19, and 21 were tested in early 2006.

3.2.1 AHU 17

AHU 17 serves the first floor emergency room and radiology, which are critical areas. Figure 2 summarizes the 2006 data for AHU 17.

The AHU 17 data indicate fungal and bacterial percent differences from OSA to ISA greater than 74%, with the exception of total fungi (spore traps), which is shown as a difference of 1%. Percent differences between before filter and after filter concentrations also show minimum percent differences of 58%, which indicate bioaerosol removal from the air stream after it passes through the filter. The low percent differences for spore traps and culturable air sampling (25°C and 37°C samples) between OSA and ISA (room) indicate indoor contamination as shown by the negative percent difference shown for the after filter to room samples. This is not an indication that the filters are inadequately filtering particles, but rather an indication of an indoor source and/or infiltration due to negative pressure contributing to the indoor concentrations of bioaerosols.

Percent differences show an increase in airborne fungal and bacterial concentrations between the locations after the filter and within the room, indicating an increase in airborne concentrations in the air for both culturable bacteria and fungi and total fungi after it leaves the AHU. Negative percent differences are identified for spore trap samples from before the filter to room and after the filter to room. The increase in concentrations as the air moves from the AHU to the room indicates the presence of an indoor source of fungi and bacteria, specifically yeasts, fungal species of *Cladosporium, Penicillium, Aspergillus*, and *Fusarium,* and bacterial species of *Staphylococcus*, *Micrococcus,* and *Sphingomonas paucimobilis*.

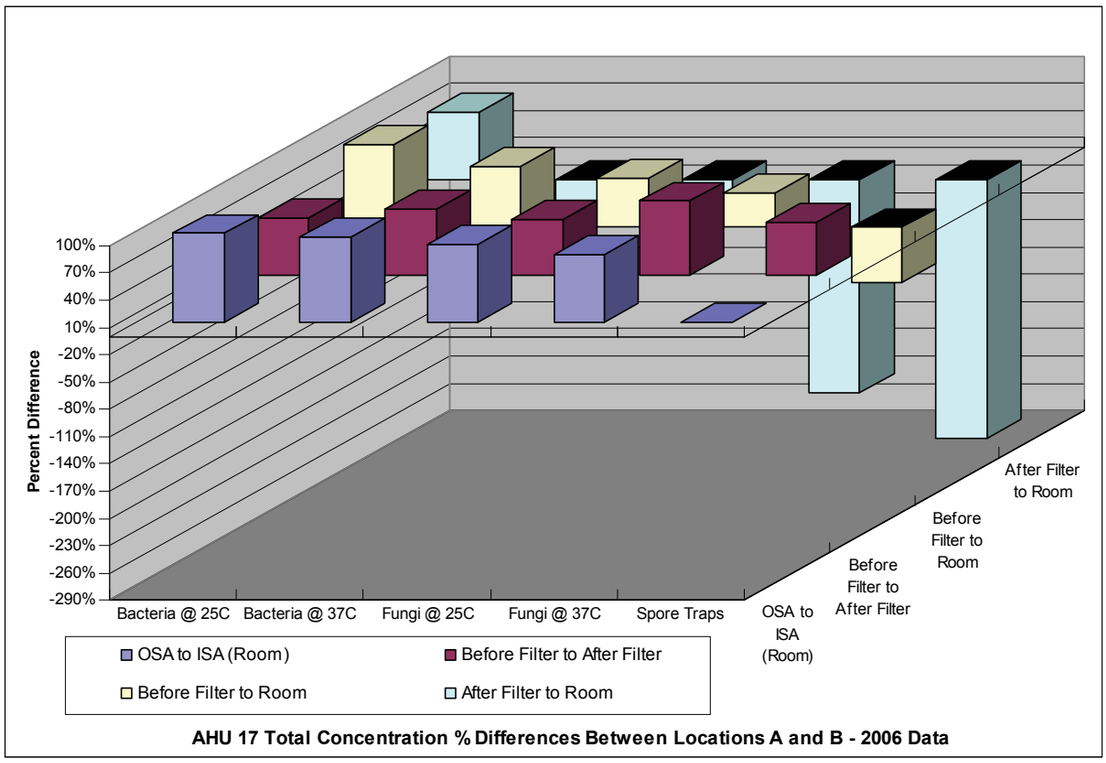


Fig. 2. AHU 17 percent differences in mean total concentrations between spaces (2006 data).
Note: A negative percent difference indicates that the indoor concentration was higher than outdoors for OSA to ISA comparisons.
Note: A black top of a bar in the graph in the 0% plane indicates a negative percent difference indicating an increase in concentration from one space to another when a decrease is expected.

3.2.2 AHU 19

AHU 19 serves the 1st Floor Administration Area, Labor and Delivery, Recovery, the Cesarean Section room, Pre-Op Area, the Intensive Care Unit, and a Rehabilitation area. All areas supplied by AHU 19 are considered critical care areas except the Administration Area. The AHU 19 data indicate percent differences that approach or are greater than 90% for fungi and bacteria between OSA and ISA. This indicates that greater than 90% of the bioaerosols in the OSA are being removed from the air stream. The AHU 19 data indicate positive percent differences signifying decreasing concentrations from before the filter to after the filter, before the filter to room, and after the filter to room. Figure 3 summarizes the 2006 data for AHU 19.

3.2.3 AHU 21

AHU 21 serves Radiology, Purchasing, Shipping and Receiving, and Plant Operations. Radiology is a critical area. The AHU 21 data indicate positive percent differences for airborne concentrations comparisons of OSA to ISA. The minimum percent difference is 54% (spore traps) for OSA to ISA comparisons and show more than 50% of the mean total concentrations of fungi and bacteria were being removed from OSA. Percent differences for bacteria incubated at 25°C were negative for the before filter to after filter, before filter to

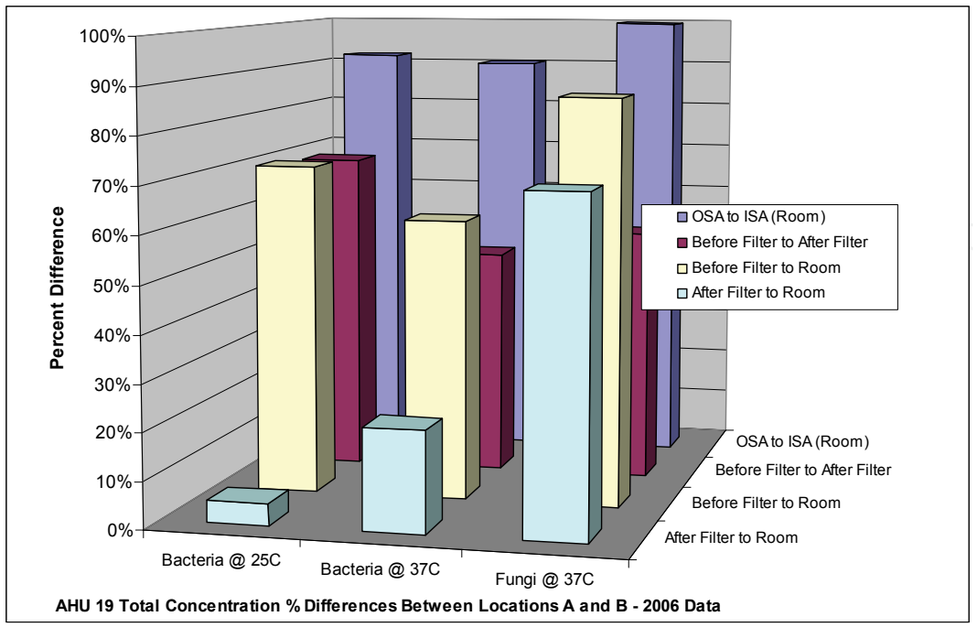


Fig. 3. AHU 19 percent differences in mean total concentrations between spaces (2006 data).
Note: A negative percent difference indicates that the indoor concentration was higher than outdoors for OSA to ISA comparisons.
Note: A black top of a bar in the graph in the 0% plane indicates a negative percent difference indicating an increase in concentration from one space to another when a decrease was expected.
Note: The 2006 AHU 19 laboratory results were not received from the analytical laboratory for spore trap and fungal samples incubated at 25°C.

room, and after filter to room sample sets, indicating an indoor source of bacteria. Percent differences for bacteria incubated at 37°C were negative for the before filter to room and after filter to room sample sets, indicating an indoor source of bacteria. Percent differences were negative for all sampling sets comparing after filter to room, indicating indoor sources of bacteria and fungi. Percent differences show an increase in airborne fungal and bacterial concentrations between air concentrations after the filter and air concentrations within the room, indicating an increase in airborne concentrations in the air for both culturable bacteria and fungi and total fungi after the air leaves the AHU.

The low percent differences for spore traps and culturable air sampling (25°C and 37°C samples) between OSA and ISA (room) indicate indoor contamination as shown by the negative percent difference shown for the after filter to room samples. This indicates an indoor source or infiltration due to negative building pressure as opposed to inadequate particle filtration by the AHU filters. The increase in concentrations as the air moves from the AHU to the room indicates the presence of an indoor source of fungi and bacteria, specifically fungal species of *Rhodotorula, Penicillium, Aspergillus, Verticillium, Nigrospora, Paecilomyces, Cladosporium, Engyodontium, Rhizopus*, and *Scytalidium,* and bacterial species of *Acinetobacter, Chryseomonas, Pseudomonas, Tatumella,* and *Staphylococcus.* Table 4 summarizes the 2006 data for AHU 21.

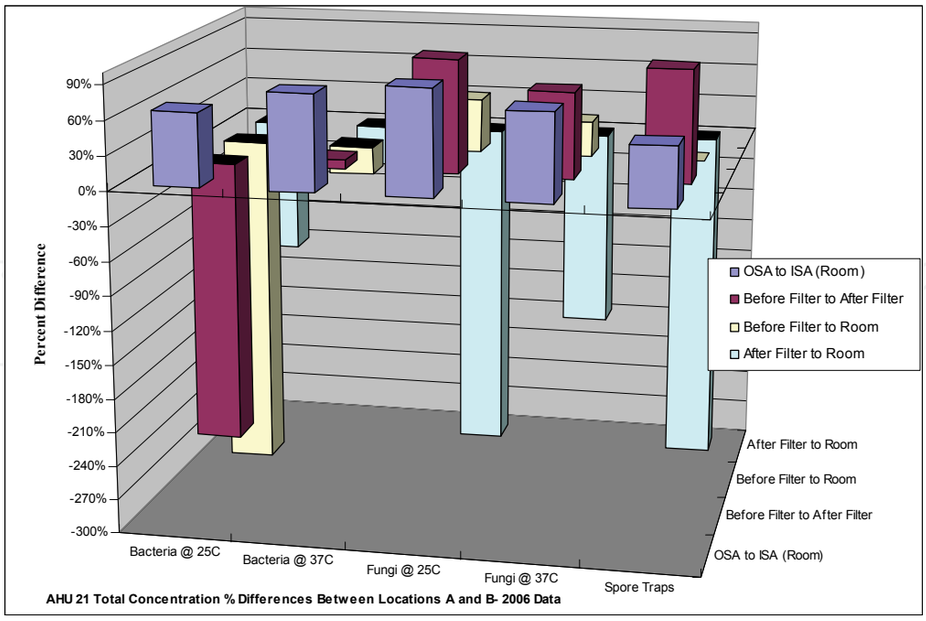


Fig. 4. AHU 21 percent differences in mean total concentrations between spaces (2006 data).
Note: Negative scale was truncated at -300% for simplicity in graphical presentation.
Note: A negative percent difference indicates that the indoor concentration was higher than outdoors for OSA to ISA comparisons.
Note: A black top of a bar in the graph in the 0% plane indicates a negative percent difference indicating an increase in concentration from one space to another when a decrease was expected.

3.3 Air sampling indicators of indoor iontamination

Indoor vs. outdoor comparisons of fungi and bacteria are used to document the presence or infer the absence of indoor, biologically derived contamination. (Macher 1999) A substantial number of bacteria and fungi are capable of spreading via the airborne route in hospitals. (Eickhoff 1994) The presence of contamination in dust or on surfaces or water is often considered de facto evidence of human exposure to fungal aerosols. (Burge 2000)

When evaluated as total concentrations, the air sampling results generally indicate lower concentrations of fungi and bacteria indoors compared to outdoors. Unless the genera and species have been identified total counts merely indicate gross numbers and indoor vs. outdoor comparisons are not meaningful. (Macher 1999; Weber and Page 2001) An investigator cannot make meaningful indoor vs. outdoor comparisons unless the genera and species found indoors and outdoors have been identified. (Macher 1999) For this project, culturable air samples were analyzed at the species level so that meaningful comparisons could be made.

When evaluated according to genera, air sampling results indicate the potential presence of indoor contamination sources for both bacteria and fungi. Air sampling indicators of an indoor source were identified in the space controlled by each AHU. Indicators of indoor contamination were defined as:

1. Potential indoor biological source (indoor mean concentration > outdoor mean concentration [tested by the ANOVA, $\alpha = 0.05$, with differences identified by SNK Post Hoc Grouping] or the organism was identified in the indoor air samples but not identified in the outdoor reference samples ($n \geq 12$) for the space controlled by each AHU),

2. Meets the criteria of 1 above (a potential indoor biological source) and is a confirmed indoor contamination source via surface sampling in the space controlled by each AHU,
3. Meets the criteria of 1 above (a potential indoor biological source) and the organism was not identified in all outdoor air samples ($n \geq 48$), indicating an indoor source of air contamination, and
4. Meets the criteria of both 2 and 3 above, confirming an indoor source of air contamination.

Air sampling identified indicators of indoor contamination within each space investigated in both the 2005 and 2006 data. While indoor bacterial sources are expected within occupied buildings, the species of fungi found in indoor and outdoor air should be similar. (Weber and Page 2001) Outdoor air samples serve as the primary comparison to indoor bioaerosol samples and the types of fungi present indoors should not be significantly different from the outdoor environment. (Spicer and Gangloff 2000) To determine if the indoor and outdoor bioaerosol profiles were similar, the means of the sampling data were tested for biodiversity. The numbers of air sampling indicators identified per AHU from the 2005 air sampling data are shown in Figure 5.

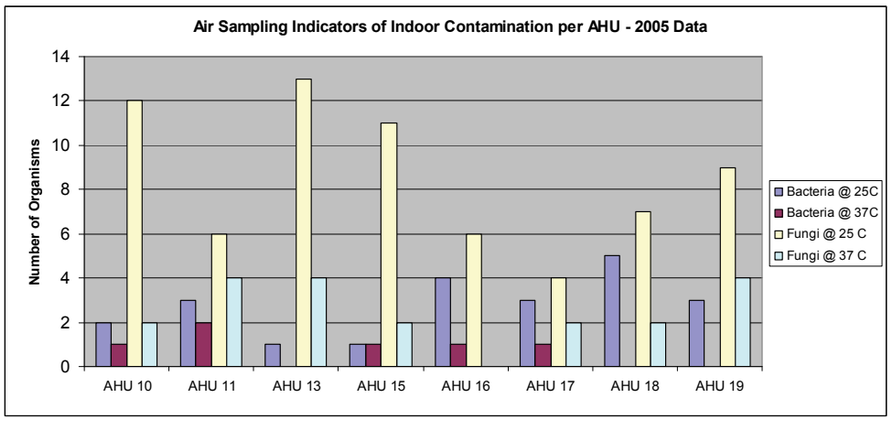


Fig. 5. Number of Air Sampling Indicators of Indoor Contamination per AHU.

Air sampling indicators identified per AHU from the 2006 air sampling data are shown in Figure 6.

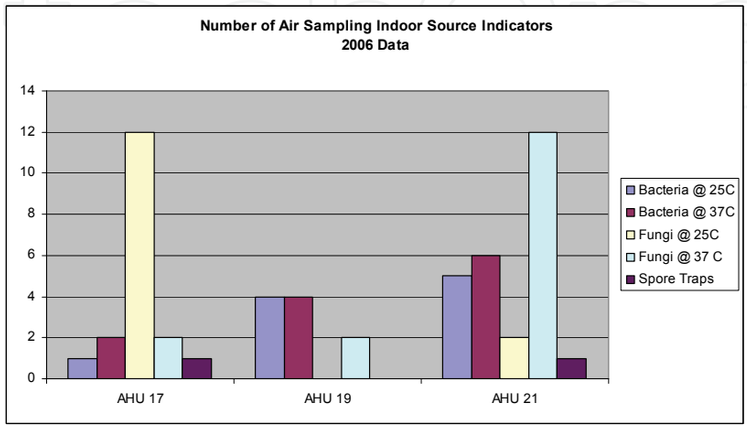


Fig. 6. Number of air sampling indicators identified via air sampling (2006 data).

Figures 7 and 8 illustrate the percentage of air sampling indicators discussed in the **Air Sampling Indicators of Indoor Contamination section** that were confirmed via surface sampling or not detected in the outside air reference samples, indicating an indoor source of contamination.

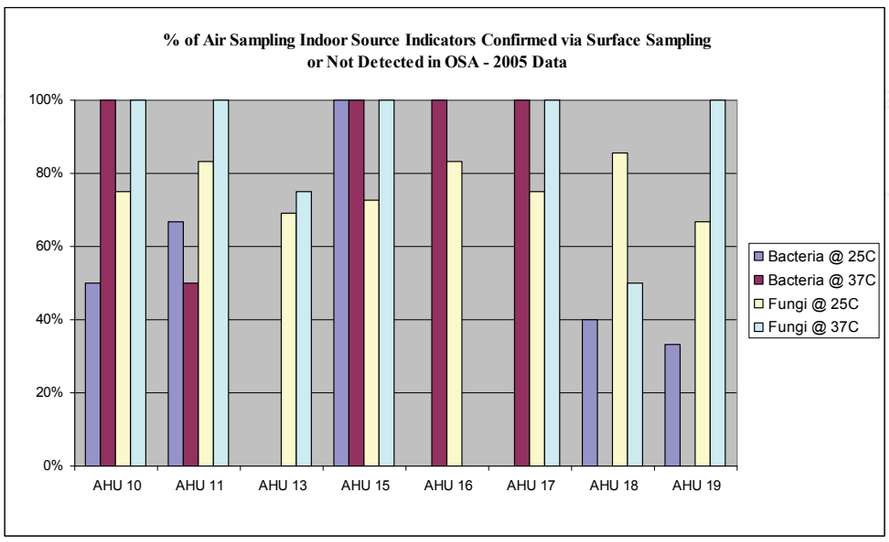


Fig. 7. Percentage of air sampling indoor source indicators confirmed via surface sampling or not detected in the OSA.

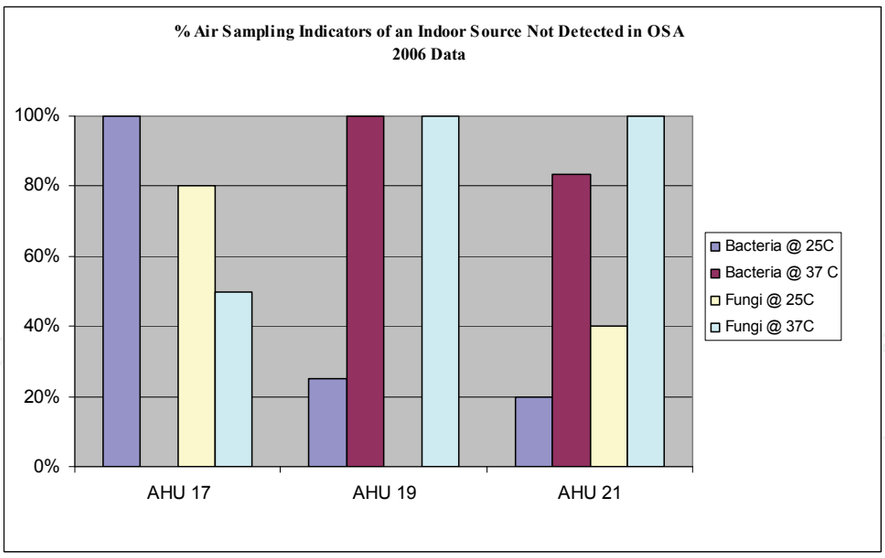


Fig. 8. Percentage of air sampling indoor source indicators not detected in the OSA.

Indicators of indoor contamination that were not identified in outdoor air samples or were identified in indoor samples were found within the space controlled by each AHU investigated. Indicators of indoor contamination identified on surface sampling are associated with the building while indicators not identified in the outdoor air are likely to be associated with the building, building occupants, or another indoor source.

3.4 Tests for biodiversity

The Spearman's Rank Correlation (SRC) is a non-parametric statistical test for comparing bioaerosol samples from separate environments. SRC is used to assess the similarity of the genera and species of culturable fungi and bacteria in air or source samples and the types of fungal spores in spore-trap samples. Data from a reference site (OSA) can be compared to data from a test site (ISA). Mean concentrations of multiple samples from each sampling location are preferred for use in the calculations for biodiversity. (Macher 1999) Indoor air sampling locations were identified broadly as the space under the environmental control of each AHU investigated. Several samples were taken from each sampling area (space under the environmental control of the AHU) so that mean concentrations could be analyzed statistically. The SRC test for biodiversity allows inferences to be made based upon whether the bioaerosol profile of one location is statistically similar to the bioaerosol profile of another. For fungi, indoor and outdoor profiles should be statistically similar. (Dillon, Heinsohn et al. 1996; Macher 1999; Spicer and Gangloff 2000) For bacteria, however, it is not unusual to have differences in biodiversity, as many bacteria are human shed. (Macher 1999)

The mix of airborne fungal species indoors should be similar to that found in the outdoor air. (Weber and Page 2001) In assessing indoor air quality with regards to airborne fungi, the types of fungi present in the indoor environment should not be significantly different from the outdoor environment. Similarity indicates that the building is not promoting or amplifying the growth of microorganisms. (Spicer and Gangloff 2000) A zero value was used as a replacement for microorganisms that were not detected in a sample. (Spicer and Gangloff 2000)

The biodiversity can be measured either as a combined aerobiological profile of fungi and bacteria, or separately. Here, bacteria and fungi are considered separately. (Macher 1999) Indoor source aerosols tend to be dominated by the readily released spores of *Aspergillus* and *Penicillium* species. (Burge 2000) The culturable air sampling results of *Aspergillus* and *Penicillium* species (at both 25°C and 37°C) were tested to determine if indoor vs. outdoor species identified were similar. Table 2 summarizes the SRC test results for biodiversity. Tests for biodiversity were conducted with SPSS 14 Statistical Software. The level of significance was prescribed as $\alpha = 0.05$. A significant correlation ($p\text{-value} \leq \alpha \leq 0.5$) indicates that there is statistical evidence that the biodiversity of the indoor air is similar to the biodiversity of the OSA. Similarity indicates that the building is not promoting or amplifying the growth of microorganisms, while dissimilarity indicates that the biodiversity of indoor and OSA are independent and that the indoor environment is amplifying the growth of microorganisms. (Dillon, Heinsohn et al. 1996; Macher 1999; Spicer and Gangloff 2000) Similarities in the biodiversity of indoor and outdoor air are unlikely to have occurred by chance, and at the $\alpha = 0.05$ (5%) level of significance, the result could have occurred by chance one time in 20. Statistical techniques evaluate an observed difference in view of its precision to determine with what probability it might have arisen by chance (the level of significance). Values with a low probability of occurring by chance are called statistically significant and are considered to represent a real effect (e.g. difference or similarity in biodiversity). (Dillon, Heinsohn et al. 1996; Conover 1999; Macher 1999) The SRC applied to bioaerosol samples in the tests for differences between OSA and ISA biodiversity at the species level for culturable bacteria and fungi and at the genus level for spore traps.

The 2005 data show consistent dissimilarity between the bioaerosol profiles of ISA and OSA for species of *Penicillium* and *Aspergillus* at both 25°C and 37°C (86% dissimilar). For AHUs 16, 17, 18, and 19, bacterial and fungal biodiversities are similar to OSA. Yet, the biodiversity of *Penicillium* species and *Aspergillus* species analyzed independently are consistently dissimilar (86% dissimilar) indicating indoor amplification. Spore traps consistently showed 100% similarity because only non-culturable fungi were analyzed at the genus level, masking differences in species present in OSA and ISA. This is an expected occurrence with spore trap analysis because spore traps analysis is not sufficient for identification at the species level. For the 2005 data, the biodiversity of bacteria between OSA and ISA were consistently similar (75% similar). The tests for biodiversity indicate that the diversity of bacterial taxa identified indoors were consistently dissimilar to the bacterial taxa identified outdoors in the space controlled by AHUs 10, 11, 13, and 15.

The 2006 data indicate that the biodiversity between OSA and ISA was dissimilar for the space under the environmental control of AHUs 17, 19, and 21. For the 2006 data, the biodiversity of bacterial tests were consistently dissimilar (83% dissimilar). This may be due to differences in seasons that the tests were conducted. The 2005 samples were taken in summer and the 2006 samples were taken in winter. During winter, OSA bioaerosol concentrations may be lower due to less than ideal growth conditions. Conversely, indoor bioaerosol concentrations of bacteria may be similar year-round due to the presence of human shed sources of bacteria.

4. Sampling interpretation summary

Allergic reactions to indoor allergens [including fungi and bacteria] can produce inflammatory conditions of the eyes, nose, throat, and bronchi. A substantial number of bacteria and fungi are capable of spreading via the airborne route in hospitals. (Eickhoff 1994) Contaminated ventilation or air conditioning systems have been implicated in nosocomial outbreaks, via infective aerosols, dust, and colonized filters. (Eickhoff 1994) Biofilms or slime on HVAC system pan or coil surfaces is an indicator of microbiological amplification. (Pope, Patterson et al. 1993) The growth of microorganisms downstream from the cooling coils can be promoted by water droplets being blown off coil surfaces and into the air. (Pope, Patterson et al. 1993; CDC 2003) Poorly designed HVAC systems may provide for amplification of fungi and *Actinomycetes* in wet niches of the system. (Pope, Patterson et al. 1993; CDC 2003) In a hospital with high efficiency filters, airborne fungal spores reflect incomplete filtration, infiltration of outside air, and shedding of adherent spores from indoor growth. (Rhame 1991) Nosocomial aspergillosis occurs in direct proportion to the mean ambient hospital airborne spore content. (Rhame 1991) Mini-bursts of spores occur from disturbance of settled spores in dust, shedding spores from clothes or sources such as indoor growth. (Rhame 1991) Immunocompromised persons, children and the elderly are at risk of from exposure to infectious microorganisms. (Boss and Day 2003) In general, the filters show that total quantities of aerobiological contaminants are being removed from the air stream. See the filter discussion in the **Total Outside Concentrations vs. Total Indoor Concentrations (2005 Data)** section. However, both surface and air samples indicate the presence of indoor microbiological amplification, which is problematic in the indoor environment.

Results for Spearman's Rank Correlation Testing for Biodiversity									
AHU	**10**	**11**	**13**	**15**	**16**	**17**	**18**	**19**	**21**
2005 Data									
Bacteria @ 25°C	D	D	S	D	S	S	S	S	Not tested in year 1.
Bacteria @ 37°C	S	S	D	S	S	S	S	S	
Fungi @ 25°C	D	S	D	D	S	S	S	S	
Fungi @ 37°C	D	S	D	D	S	S	S	S	
Spore Traps	S	S	S	S	S	S	S	S	
Aspergillus @ 25°C	D	D	D	D	D	D	D	D	
Penicillium @ 25°C	D	D	D	D	D	S	D	D	
Aspergillus @ 37°C	D	S	D	D	*	D	S	S	
Penicillium @ 37°C	*	D	D	D	*	D	D	D	
2006 Data									
Bacteria @ 25°C	Not tested in year 2.					D	Not tested in year 2.	D	D
Bacteria @ 37°C						D		D	S
Fungi @ 25°C						S		**	S
Fungi @ 37°C						D		*	D
Spore Traps						S		**	S
Aspergillus @ 25°C						*		*	*
Penicillium @ 25°C						S		*	*
Aspergillus @ 37°C						*		*	*
Penicillium @ 37°C						*		*	*

S indicates that the aerobiological profiles between indoor and outdoor air are "similar" and not independent (i.e. the populations appear to be related or the samples could have been drawn from the same environment), ($\alpha \leq 0.05$, Spearman's Rank Correlation). (Dillon, Heinsohn et al. 1996; Macher 1999)

D indicates that the aerobiological profiles between indoor and outdoor air are "dissimilar" and independent (ie. The populations appear to be unrelated and drawn from separate environments). (Macher 1999; Dillon, Heinsohn et al. 2005)

* indicates that the Spearman's Rank Correlation test could not be performed due to indoor concentration data that were constant (zero) or the number of species identified for each test was not sufficient to warrant testing via Spearman's Rank Correlation. (Spicer and Gangloff 2000)

** Note: Sample results were not received from the analytical laboratory.

Note: One tailed test for correlation. Significant correlation indicates that the indoor and outdoor biological profiles are similar. (Conover 1999; Macher 1999)

Table 2. Spearman's Rank Correlation Test for Biodiversity between outdoor and indoor sampling locations. (Significance level $\alpha \leq 0.05$)

4.1 Fungal samples

The mix of airborne fungal species indoors should be similar to that found in the outdoor air. Fungal organisms identified indoors, that are not present in the outdoor air or control locations, suggests the presence of an amplifier (growth site) for that species in the building. (Macher 1999; Weber and Page 2001) Most fungi can become opportunistic pathogens in a severely immunocompromised patient. (Weber and Page 2001) Airborne fungi within the hospital setting are especially dangerous because antifungal therapy is still rather ineffective. (Kalliokoski 2003) Although average air concentrations of total culturable fungi indoors were consistently lower than those found outdoors, many types of fungi identified indoors were not found in outdoor reference samples; especially within the species of *Aspergillus* and *Penicillium*. (Weber and Page 2001) The biodiversity of species of *Penicillium* and *Aspergillus* between indoor and outdoor air was consistently dissimilar for sampling results within the sampling space controlled by each air handling unit. Species of fungi identified within the hospital in air and on surfaces that are associated with airborne transmission or NI are *Penicillium*, *Aspergillus*, *Rhizopus*, *Acremonium*, and *Fusarium*. (CDC 2003)

Indicator organisms identified via air sampling in the hospital are *Aspergillus versicolor, A. flavus*, *A. fumigatus*, species of *Fusarium* and *Penicillium*, and yeasts. (Macher 1999) Some indicator organisms identified on indoor surfaces within the Hospital are species of *Aspergillus*, *Chaetomium, Stachybotrys*, *Penicillium*, *Fusarium*, *Acremonium*, *Trichoderma*, and yeasts. The presence of indicator organism contamination on surfaces indicates long-term or severe moisture problems. (Boss and Day 2003) Indicator organisms were identified on surfaces within the space controlled by each AHU investigated in 2005.

Exposure to fungi actively growing indoors may present unusual health risks even when total fungal concentrations are higher outdoors. (Macher 1999) Exposure to damp indoor environments and the presence of molds in damp indoor environments are associated with asthma symptoms in sensitized asthmatic persons. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) Serious respiratory infections resulting from exposure to *Aspergillus* species and *Fusarium* species are common in persons who are immunocompromised. It is likely that many of these fungal infections are contracted through contact with fungi in indoor environments, because poor health conditions limit people with severely impaired immune systems to spend most of their time indoors. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) The lungs of persons with chronic pulmonary disorders such as cystic fibrosis, asthma, and chronic obstructive pulmonary disorder may become colonized and potentially infected with *Aspergillus* species. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) Healthy persons exposed to damp or moldy indoor environments report that they are more prone to respiratory infections, including the common cold, sinusitis, tonsillitis, otitis, and bronchitis. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) Fungi have become the largest cause of occupational diseases among healthcare workers in Finland. (Kalliokoski 2003)

Indoor source aerosols tend to be dominated by the readily released spores of *Aspergillus* and *Penicillium* species (Burge 2000), which produce large numbers of spores that are easily released into the air. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) Indoor exposure to *Aspergillus* and *Penicillium* species spores has been shown to be associated with an increased risk of allergic sensitization in children (Wilson, Holder et al. 2004) and are chiefly involved in the genesis of asthma and allergic alveolitis (pulmonitis due

to hypersensitivity). (Perdelli, Christina et al. 2006) Bronchial asthma is frequently provoked by airborne fungal spores belonging to the genera *Aspergillus* and *Penicillium*. (Smith 1990) Species of *Aspergillus* and *Penicillium* are considered indicator organisms that may signal unwanted moisture intrusion and/or a potential for health problems. (Macher 1999) As such, the biodiversity of both *Penicillium* and *Aspergillus* species was tested to determine whether the outdoor air was the primary source for the fungi identified in the indoor air. (Macher 1999)
Aspergillus and Penicillium species are two of the most ubiquitous fungi known. Large quantities of fungal spores are produced when these fungi are actively growing. During germination, large quantities of spores are produced, and when sporulation occurs, several thousand spores may be disseminated per cubic meter of air. (Wenzel 1997) *Penicillium* and *Aspergillus* spores are sphere-like, measuring from approximately 2-5 micrometers in diameter and can be suspended very easily in the air. (Wenzel 1997; Straus 2004) Spores of *Penicillium* species often cannot be distinguished via microscopic examination from spores of *Aspergillus* species and vice versa. (Stetzenbach and Yates 2003) Once suspended, they may remain suspended for prolonged periods, and when those spores settle, they can contaminate any surface in contact with air. (Wenzel 1997) Once inhaled, these spores travel through the airways into the lower regions of the lungs, leading to the potential development of respiratory symptoms. (Straus 2004)

4.1.1 Aspergillus species

The presence of *Aspergillus* species in health-care environments is a substantial extrinsic risk factor for opportunistic invasive aspergillosis (invasive aspergillosis being the most serious form of the aspergillosis). (CDC 2003) The presence of *Aspergillus* contamination and/or growth within a health care facility is of particular concern due to the presence of immunocompromised persons. Causative agents of aspergillosis are *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus*, and *Aspergillus nidulans*. (CDC 2003) Airborne concentrations of *Aspergillus* species at or below 0.1 cfu/m^3 have been recommended for the prevention of nosocomial aspergillosis. (Weber and Page 2001) Low concentrations of *A. fumigatus* and *A. flavus* have been associated with nosocomial aspergillosis in immunocompromised patients. (Arnow, Sadigh et al. 1991) Among immunosuppressed patients in general, invasive aspergillosis remains a serious complication and may be lethal. (Perdelli, Christina et al. 2006)
The genus *Aspergillus* is one of the most ubiquitous fungi known. Large quantities of fungal spores are produced when actively growing. The *Aspergillus* spores are inhaled easily because of their small aerodynamic size and can easily reach and colonize the upper respiratory tract, including paranasal sinuses and terminal airways. (Wenzel 1997) Nosocomial pulmonary and disseminated aspergillosis arises from inhalation of fungal spores. (Rhame, Streifel et al. 1984) The use of powerful new chemotherapy protocols for malignancies and certain immunologic disorders and the increasing use of organ transplantation are risk factors for nosocomial aspergillosis. Patients with acute or chronic myelogenous leukemia and AIDS are particularly susceptible to nosocomial aspergillosis. In the transplant population and in patients with aplastic anemia, *Aspergillus* has emerged as a major cause of death. (Wenzel 1997)
Nosocomial aspergillosis is primarily established when an immunocompromised host inhales fungal spores present in the air. (Rhame, Streifel et al. 1984; Wenzel 1997) Any dust-generating activity, such as maintenance of ventilation systems, cleaning, vacuuming, and dry mopping, can render *Aspergillus* spores airborne and potentially cause outbreaks of

nosocomial aspergillosis. The achievement of a spore-free air within an area or ward of a hospital may not be sufficient to eradicate nosocomial aspergillosis because of "non-ward" sources of *Aspergillus* within the hospital, such as radiology, radiation therapy units, and other areas visited by patients where engineering and environmental controls may not be as stringent. (Wenzel 1997) Fungi actively growing indoors compounds the problem associated with the prevention of nosocomial aspergillosis.

Species of *Aspergillus* found indoors should be similar to species identified outdoors. With the exception of AHU 11, all AHUs have filters of 90% efficiency or greater. 90% and 95% filters are considered high efficiency (Boss and Day 2003) and rated to remove 90% of particles from 1-10 micrometers and almost all particles greater than 10 micrometers in size. (ASHRAE 1992; ASHRAE 1999) Due to the presence of high efficiency filters in the AHUs investigated (except AHU 11), indoor concentrations should be statistically lower than outdoor concentrations and organisms not detected outdoors should not be detected indoors (Weber and Page 2001) (with the exception of indoor-source bacteria (Macher 1999)). However, many species of *Aspergillus* were detected indoors and not outdoors or indoor concentrations were greater than or not statistically different than outdoor concentrations, indicating an indoor source or infiltration.

The average concentration of *A. fumigatus* for culturable fungal air samples at 25°C was 0.50 cfu/m3 (n=14 indoors, n=38 outdoors) in the space controlled by AHU 15. The biodiversity of species of *Aspergillus* between indoor and outdoor air was consistently dissimilar for sampling results (incubated at 25°C) within the sampling space controlled by each air handling unit, indicating the presence of indoor fungal amplifiers; the building appears to be promoting or amplifying the growth of species of *Aspergillus*. (Macher 1999; Spicer and Gangloff 2000; Weber and Page 2001)

Of particular concern in a hospital setting are the presence of thermotolerant fungi, including *A. fumigatus* and *A. flavus*. Thermotolerant fungi are of primary concern in healthcare facilities, since they can cause infection in at-risk patients, even when concentrations are very low. (Page and Trout 2001) Concentrations of thermotolerant *A. fumigatus* (incubated at 37°C) were 1.17 (n=12 outdoors, n=6 indoors) and 3.50 cfu/m3 (n=12 indoors, n=22 outdoors) in the spaces controlled by AHUs 13 and 15, respectively. Indoor concentrations of *A. flavus* were not statistically different than outdoor concentrations in the spaces controlled by AHUs 11 and 17 for samples incubated at 25°C and AHUs 11, 17, and 18, for samples incubated at 37°C. This is an expected condition within the space controlled by AHU 11 due to the absence of final filters. This suggests the presence of an indoor source of *A. flavus* or outdoor air infiltration within the spaces controlled by AHUs 17 and 18. Indoor *A. flavus* concentrations detected in the space controlled by AHUs 10 and 19 were lower than OSA concentrations (statistically significant difference at the $\alpha = 0.05$ level of significance). The biodiversity of species of thermotolerant *Aspergillus* between indoor and outdoor air was consistently dissimilar for sampling results within the sampling space controlled by AHUs 10, 13, 15, and 17, indicating the presence of indoor fungal amplifiers; the building appears to be promoting or amplifying the growth of species of *Aspergillus*. (Macher 1999; Spicer and Gangloff 2000; Weber and Page 2001)

Indoor concentrations for culturable *Aspergillus* species incubated at 25°C (*A. flavus, A. sydowii, A. nidulans, A. niger, A. fumigatus, A. flavipes , A. sclerotiorum, A. terreus, A. versicolor*) that exceeded indoor concentrations, were not statistically different to outdoor concentrations, or not detected in the outdoor reference samples were identified in the space controlled by AHUs 11, 13, 15, 16, 17, 18, and 19. Indoor concentrations of thermotolerant

species of *Aspergillus* (*A. flavus, A. sydowii, A. niger, A. fumigatus, A. terreus)* that exceeded indoor concentrations, were not statistically different to outdoor concentrations, or not detected in the outdoor reference samples were identified in the space controlled by AHUs 10, 11, 13, 15, 17, and 18.

Potentially hazardous concentrations of *A. fumigatus* were identified in the spaces controlled by AHUs 13 and 15. (Weber and Page 2001) Moisture intrusion via infiltration, leaks, inadequate HVAC control, etc. has provided a chronically moist indoor environment ideal for fungal growth. Air infiltration due to negative building pressurization allows the introduction of unfiltered air into the hospital. Air samples indicated the presence of indoor fungal reservoirs/amplifiers within the spaces controlled by all AHUs investigated. Air and surface sampling indicated microbial growth, dissemination and, hence, occupant exposure, from the indoor microbial reservoirs. (Weber and Page 2001) An indoor environment has been created in which immunosuppressed or allergic patients within the Hospital are not fully protected against the risk of infection and the allergenic effects of *Aspergillus* species (Perdelli, Christina et al. 2006) and otherwise healthy persons may suffer exacerbation of allergies and be prone to increased incidences of respiratory infections, including the common cold, sinusitis, tonsillitis, otitis, and bronchitis. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004)

General controls for prevention of nosocomial aspergillosis are: air filtration, positive pressurization, avoidance of dust-generating activities, attention to non-filtered air infiltration, protection of immunocompromised patients who enter areas without highly filtered air, and isolation of hospital construction. (Wenzel 1997) Highly filtered air is essential to preventing person-to-person and environmentally related infections. (Boss and Day 2003) However, it is important to note that the use of highly filtered air conditioning systems does not provide complete protection against fungi actively growing or infiltrating into a facility. (Perdelli, Christina et al. 2006) Nosocomial aspergillosis has been associated with poorly maintained and/or malfunctioning HVAC systems. (CDC 2003)

4.1.2 Penicillium species

As with *Aspergillus* species, the genus *Penicillium* is one of the most ubiquitous fungi known and one of the most commonly isolated molds from contaminated buildings. With *Penicillium* species the main cause for concern is allergic disease, as infections due to *Penicillium* species are rare. Indoor concentrations of *Penicillium* species greater than outdoor concentrations are associated with negative health effects in humans. *Penicillium* species has been correlated with allergic asthma, allergic alveolitis, atopy, increased lower respiratory infections in children during the first year of life, and wheezing. (Straus 2004)

Inhalation of *Penicillium* species spores has been shown to provoke immediate and delayed-type asthma in individuals already sensitized to *Penicillium*. (Straus 2004) Infants with high risk for the development of asthma (e.g. due to premature birth and/or ethnicity) may be at significant risk for persistent cough and wheeze when exposed to *Penicillium* species spores. *Penicillium* species have been shown to cause allergic alveolitis due to exposure from a faulty installation of a HVAC system. (Straus 2004) *Penicillium* is a large group of fungi valued as producers of antibiotics. *Penicillium* may cause allergic reactions, exacerbate asthma, and cause other adverse health effects when dispersed through air. (Boss and Day 2003; Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) The blue-green molds of *Penicillium* are common contaminants of indoor environments. Inhalation of

spores is the major route of entry. *Penicillium* species have been associated with asthma and hypersensitivity pneumonitis (Weber and Page 2001) and can cause NI in the immunocompromised host. (Fox, Chamberlin et al. 1990; Walsh and Groll 1999; CDC 2003) *Penicillium* species spores have been shown to be associated with an increased risk of allergic sensitization in children (Straus 2004) and are chiefly involved in the genesis of asthma and allergic alveolitis (pulmonitis due to hypersensitivity). (Perdelli, Christina et al. 2006) One 2.5 cm diameter colony of *Penicillium* species can produce 400,000,000 spores that can become airborne and generate increased concentrations of airborne spores. (Hitchcock, Mair et al. 2006)

The biodiversity of *Penicillium* species between ISA and OSA was consistently dissimilar for sampling results (incubated at 25°C) within the sampling space controlled by each AHU (except AHU 17), indicating the presence of indoor fungal amplifiers; the building appears to be promoting the growth of species of *Penicillium*. (Spicer and Gangloff 2000) Thermotolerant fungi are of primary concern in healthcare facilities, since they can cause infection in at-risk patients, even when concentrations are very low. (Weber and Page 2001) The biodiversity of species of thermotolerant *Penicillium* between indoor and outdoor air was consistently dissimilar for sampling results within the sampling space controlled by each AHU (except AHUs 10 and 16 because tests could not be performed), indicating the presence of indoor fungal amplifiers; the building appears to be promoting or amplifying the growth of species of *Penicillium*. (Macher 1999; Spicer and Gangloff 2000; Weber and Page 2001)

Species of *Penicillium* found indoors should be similar to species identified outdoors. With the exception of AHU 11, all AHUs have filters of 90% efficiency or greater and should remove greater than 90% of particles between 1 and 10 micrometers in size. Due to the presence of high efficiency filters in the AHUs investigated (except AHU 11), indoor concentrations should be statistically lower than outdoor concentrations and organisms not detected outdoors should not be detected indoors (with the exception of indoor-source bacteria). (Weber and Page 2001) However, many species of *Penicillium* were detected indoors and not outdoors or indoor concentrations were greater than or not statistically different than outdoor concentrations. Indicators of indoor contamination of *Penicillium* species were identified in the space controlled by each AHU.

Indoor airborne concentrations of culturable Penicillium species incubated at 25°C (P. citrinum, P. chrysogenum, P. corylophilum, P. decumbens, P. duclauxii, P. funiculosum, P. glabrum, P. implicatum, P. janthinellum, P. oxalicum, P. pinophilum, P. purporogenum, P. sclerotiorum, P. variabile, P. waksmani) that exceeded or were similar to outdoor concentrations or not detected in the outdoor reference samples were identified in the space controlled by each AHU. Indoor airborne concentrations of thermotolerant (incubated at 37°C) species of Penicillium (P. citrinum, P. chrysogenum, P. decumbens, P. funiculosum, P. janthinellum, P. oxalicum, P. pinophilum, P. simplicissimum) that exceeded or were similar to outdoor concentrations or not detected in the outdoor reference samples were identified in the space controlled by each AHU, with the exception of AHU 10 where no thermotolerant species of Penicillium were identified.

4.1.3 Yeasts

Yeasts are found in a variety of natural habitats or organic substrates such as plant leaves, flowers, soil, and salt water. Some yeasts are part of the normal human flora. Although yeasts may be part of the normal human flora, they were detected on indoor surfaces.

Therefore, it can be concluded that the yeasts identified as indicators of indoor contamination were most likely from an indoor contamination source. (Macher 1999) The presence of yeasts actively growing indoors is of concern, as yeasts are considered an indicator organism and can cause infections in the immunocompromised host. Some yeasts are reported to be allergenic, and may cause problems in individuals with previous exposure and developed hypersensitivities. Yeast infections are among the most common fungal infections in humans. Their form ranges from localized cutaneous or mucocutaneous lesions, to fungemia or disseminated systemic mycoses. (AerotechP&K 2006) Indoor concentrations of yeasts exceeded outdoor concentrations or were detected indoors and not outdoors in the spaces controlled by AHUs 10, 13, 15, and 19. See indicators of indoor contamination for both 2005 and 2006 data. Yeasts were identified via surface sampling within the spaces controlled by AHUs 10, 11, 13, 15, 16, 17, 18, and 19.

4.2 Bacterial samples

Like fungi, bacteria actively growing indoors can release spores into the air (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004). Additionally, bacteria secrete enzymes that can act as allergens. Enzymes and spores from Gram-positive bacilli and thermophilic *Actinomycetes* have been implicated in epidemics of hypersensitivity pneumonitis and work-related asthma. Concentrations of bacteria associated with sensitization or provoking human allergic reactions are unknown. (Pope, Patterson et al. 1993) Bacteria are known to cause diseases either as pathogens or as opportunistic pathogens in the immunocompromised host. (Boss and Day 2003) Environmental bacteria also grow in all wet spaces and are found in most cases where there is fungal growth. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) Some bacteria that are common in outdoor air may penetrate to building interiors and may also grow indoors. (Macher 1999) Unlike fungi, bacteria have natural reservoirs indoors (primarily humans), and total bacterial concentrations are often higher indoors than outdoors. (Macher 1999)

The bacterial organisms identified (incubated at 25°C) as indicators of an indoor source were Acinetobacter lwoffi, Gram (+) cocci, Micrococcus luteus, Micrococcus species, Staphylococcus species (S. auricularis, S. capitus, S. epidermis, S. hominis, S. hyicus, S. warneri, and S. xylosus) which are human-shed bacteria. (Wilson 2005) Because these organisms are human-shed and were not identified as indoor contaminants via surface sampling, it cannot be concluded that these higher concentrations of indoor-source bacteria detected via air sampling were the result of building-related sources of bacterial contamination. (Macher 1999)

The bacterial organisms identified (incubated at 37°C) as indicators of an indoor source were Gram (-) cocci, *Pseudomonas aeruginosa, Pseudomonas stutzeri, Rhizobium radiobacter, and Tatumella ptyseos*. Gram (-) bacteria are usually not present on the skin (with the exception of *Acinetobacter* species). *Pseudomonas aeruginosa* can be found on skin, but is also considered an environmental organism and may be associated with building contamination and/or infiltration. (Wilson 2005) *Pseudomonas stutzeri and Rhizobium radiobacter* are considered environmental source organisms. The presence of airborne concentrations of *Pseudomonas stutzeri* (identified via surface sampling on a ceiling tile in the space controlled by AHU 19), and *Rhizobium radiobacter* indicates the presence of an indoor environmental source (amplification).

Acinetobacter species may cause NI and death in infants during periods of airborne dissemination. Environmental conditions leading to an increase in air conditioner condensate in HVAC systems may increase the risk of nosocomial infection with *Acinetobacter* species. (McDonald, Walker et al. 1998) *Acinetobacter* species have been cultured from air conditioners in a hospital nursery. (CDC 2003) *Acinetobacter lwoffi* species was identified as an indicator of an indoor source via air sampling in the space controlled by AHUs 13 and 21. *Acinetobacter* species are widely distributed in the environment and are frequently found on human skin. (Wilson 2005) *Acinetobacter* sp. are a main causative agent of pneumonia, which is a leading cause of morbidity and mortality and is the sixth most common cause of death in the United Kingdom and the Untied States. (Wilson 2005) The presence of *Acinetobacter* sp. in the air is of concern in the hospital environment. There is no indication that the indoor source of *Acinetobacter* species was associated with building contamination, but is likely associated with infiltration.

Pseudomonas species are common in the outdoor air, but rarely occur in indoor air. (Macher 1999) *P. aeruginosa* was identified as an indicator of indoor contamination in the space controlled by AHU 11. This may be due to the absence of final filters in AHU 11. Airborne infections by *P. aeruginosa* have been reported. (Kalliokoski 2003) Transmission of *P. aeruginosa* may occur through direct patient-to-patient contact, environmental contamination, or via the hands of health care workers. (Kerr, Moore et al. 1995; Beggs and Kerr 2000) Airborne *P. aeruginosa* was detected within the hospital in 2005 during a pilot study of proposed sampling equipment in the basement of the Hospital. These sampling results must be viewed as a qualitative indication of an indoor source of *P. aeruginosa*, as the equipment utilized in the 2005 pilot study was determined to be inaccurate for the determination of airborne concentrations but sufficient for qualitative identification of the bacterium at high concentrations within the space tested. *P. aeruginosa* is an environmental organism typically found on skin of people and has been isolated environmentally from soil, manure, canal water, and straw. *P. aeruginosa* is a ubiquitous soil organism that proliferates in standing water and wet and warm materials such as leaking hot water pipe insulation and showers. (Boss and Day 2003; CDC 2003; Stetzenbach and Yates 2003) *P. aeruginosa* is an opportunistic pathogen and can be especially problematic for those with cystic fibrosis and burn victims. (Boss and Day 2003; CDC 2003; Gaynes and Edwards 2005) Nosocomial infections due to *P. aeruginosa* have been associated with poorly maintained and/or malfunctioning HVAC systems. (CDC 2003) *P. aeruginosa* causes urinary tract and skin infections, septicemia (blood infections), and meningitis. (Boss and Day 2003; Wilson 2005) *P. aeruginosa* is a main causative agent of pneumonia, which is a leading cause of morbidity and mortality and is the sixth most common cause of death in the United Kingdom and the Untied States. (Wilson 2005) The presence of *P. aeruginosa* in the air is of concern in the hospital environment.

Pseudomonas oryzihabitans was identified as an indicator of indoor contamination within the space controlled by AHU 21 (2006 data). *P. oryzihabitans* is an opportunistic pathogen and is a common soil bacterium. (Freney, Hansen et al. 1988; Bendig, Mayes et al. 1989; Munro, Buckland et al. 1990; Podbielski, Mertens et al. 1990; Reina, Odgardd et al. 1990; Esteban, Valero-Moratalla et al. 1993; Lam, Isenberg et al. 1994; Lucas, Kiehn et al. 1994; Rahav, Simhon et al. 1995; Romanyk, Gonzalez-Palacios et al. 1995; Liu, Shi et al. 1996; Anzai, Kudo et al. 1997; Kiris, Over et al. 1997; Lin, Hsueh et al. 1997; Marin, Garcia de Viedma et al. 2000)

Pseudomonas stutzeri was identified as an indicator of an indoor source in the space controlled by AHU 15 (2005 data) and AHU 19 (2006 Data). It is considered an

environmental organism and has been isolated from soil, manure, canal water, and straw. It has been isolated from the respiratory tract, wounds, blood, urogenital tract, spinal and joint fluid of humans and is associated with NI. (Palleroni, Doudoroff et al. 1970; Reisler and Blumberg 1999; Taneja, Meharwal et al. 2004; Lalucat, Bennasar et al. 2006; Yee-Guardino, Danziger-Isakov et al. 2006) *P. stutzeri* surface contamination was confirmed from a ceiling tile within the space controlled by AHU 19. The presence of indoor contamination of *P. stutzeri* is of concern in the hospital environment.

Pseudomonas species are one of the most antibiotic resistant bacteria and resistant to antiseptics such as quaternary ammonium compounds. (Georgiev 1998; Boss and Day 2003) This property allows them to survive environmental conditions which are lethal to many other bacteria. (Georgiev 1998) *Pseudomonas* species in general are among the most clinically relevant healthcare associated pathogens. (CDC 2003) In general, the presence of *Pseudomonas* in a hospital setting is problematic. (Boss and Day 2003) *P. fluorscens* was identified as a contaminant in the drain pan water of AHU 19 and *P. Stutzeri* was identified as surface contamination in the space controlled by AHU 19. The presence of *Pseudomonas* species actively growing in the indoor healthcare environment is especially problematic. (Boss and Day 2003)

Rhizobium species are environmental source fungi typically found in the roots of plants and in soils. (Stetzenbach, Buttner et al. 2004) *Rhizobium radiobacter* was identified as an indicator of indoor contamination in the space controlled by AHU 11, and was not detected in the outdoor air (n = 118 outdoor air samples). This indicates the presence of an indoor source. *Rhizobium* sp. is recognized as an opportunistic human pathogen associated with NI in the immunocompromised host. (Lai, Teng et al. 2004) It is likely that the absence of final filters in the space controlled by AHU 11 prevented indoor concentrations of *Rhizobium* sp. from being removed from the air stream. It is likely that the *R. radiobacter* contamination originated from within the Hospital from an environmental source.

Staphylococcus aureus was identified in the condensate water of AHU 21 (2006 data). The presence of *S. aureus* actively growing indoors should be considered a health risk in the hospital environment. *S. aureus* produces toxins and can infect surgical wounds, develop resistance to antibiotics, and is the agent of toxic shock syndrome. *S. aureus* also produces toxins that cause food poisoning. (Boss and Day 2003) *S. aureus* was not identified in the indoor air via air sampling.

Tatumella sp. is a member of the family *Enterobacteriaceae*, which are widely distributed in soil, water, plants, and animals. (Hollis, Hickman et al. 1981; Georgiev 1998) *Enterobacteriaceae* are responsible for over half of the NI in the United States. (Hollis, Hickman et al. 1981) *Tatumella ptyseos* was identified as an indicator of an indoor source in the space controlled by AHUs 10 (2005 data) and 21 (2006 data). *T. ptyseos* is associated with NI, but there is no indication that the indoor source of *Tatumella* species was associated with building contamination.

4.2.1 Actinomycetes

Actinomycetes were once considered fungi because of their resemblance to fungi. However, these organisms are not fungi, but are bacteria. (Georgiev 1998; AerotechP&K 2006) The presence of *Actinomycetes* is rare in buildings and outdoors. The presence of *Actinomycetes* indoors may be considered an indication of an indoor environmental source (Macher 1999) and may add to the complexity of the environmental problem. (Straus 2004) The

Actinomycetes have the potential to become opportunistic, especially in immunocompromised hosts. (McNeil and Brown 1994; Georgiev 1998)
Thermophilic *Actinomycetes* are usually found in closed barns, silos, grain mills, and bagasse (sugar cane waste). *Actinomycetes* have been found in problematic or poorly maintained air conditioning ducts. (AerotechP&K 2006) Allergic respiratory disease caused by the *Actinomycetes* is referred to as farmer's lung, a hypersensitivity reaction from repeated exposure to antigens produced by the *Actinomycetes*. *Actinomycetes* may also cause other diseases such as ocular infections, periodontal disease, and abscess formations, which can infect humans. (Georgiev 1998) Several reports indicate that infections by these bacteria are not rare (especially from the *Actinomycete* genus *Nocardia*), are frequently misdiagnosed, or are under diagnosed, and that the incidence of infection is increasing. The spectrum of disease caused by *Nocardia* is broad and varies from a self-limited, asymptomatic infection to an aggressive, destructive disease resulting in death. *Nocardial* infections are commonly diagnosed in previously healthy adults with no predisposing factors. (McNeil and Brown 1994) The *Nocardiae* are frequently being recognized as emerging opportunistic pathogens; the most common underlying predispositions include organ transplantation, malignancies, use of corticosteroids, alcohol abuse, diabetes, or other debilitating factors. (McNeil and Brown 1994; AerotechP&K 2006)
Nocardioform bacilli and/or presumptive *Nocardioforms* were identified as indicators of an indoor microbiological source in the space controlled by AHUs 17, 18, and 19. *Nocardioforms* include the genus *Nocardia* and the *Nocardioform Actinomycetes*. (Georgiev 1998; Boss and Day 2003; Gibson, Gilleron et al. 2003; Stetzenbach and Yates 2003) *Nocardia* species are the *Nocardioform* most often isolated from NI. (Georgiev 1998) *Nocardia* are found in soil around the world, and the indoor concentrations of *Nocardioforms* were not identified via surface sampling and could not be associated with building contamination. (Macher 1999) *Nocardia* morphologically resembles *Actinomyces* species, and both are bacteria that are often pathogenic and opportunistic. (McNeil and Brown 1994; Georgiev 1998; Boss and Day 2003; AerotechP&K 2006)
Actinomycetes were detected indoors via air sampling and not outdoors (n=94 outdoor air samples) within the space controlled by AHU 10 and AHU 17 for the fungal samples incubated at 25°C. *Actinomycetes* were detected indoors and not outdoors (n=48 outdoor air samples) via air sampling within the space controlled by AHU 19 for the fungal samples incubated at 37°C. *Actinomycetes* were confirmed via surface sampling within the spaces controlled by AHUs 13, 15 (*Actinomyces* sp.), 16, and 17 (*Actinomycetes*-like). Therefore, it can be concluded that the airborne concentrations of *Actinomycetes* identified within the Hospital were due to an indoor source of surface contamination. (Macher 1999) The presence of *Actinomycetes* indoors is of concern in the indoor hospital environment.

5. Surface sampling interpretation and health risk model

Allergic reactions to indoor allergens can produce inflammatory diseases of the eyes, nose, throat, and bronchi, which are medical problems that come under the headings of allergic conjunctivitis, allergic rhinitis, allergic asthma, and hypersensitivity pneumonitis (extrinsic allergic alveolitis) respectively. (Pope, Patterson et al. 1993) The Health Risk Model (HRM) considers the type of microbial contamination and the type of person expected to be within a specific Hospital location. Critical care areas are areas of the Hospital where it is expected

that immunocompromised persons will be present and therefore contamination within a critical care area is given a higher weight in the overall determination of health risk.

Risk assessment is a process designed to evaluate the potential relationship that may exist between exposure to aeroallergens and a particular effect (e.g. toxic effect, allergic sensitization, infection, allergic disease). (Pope, Patterson et al. 1993) A HRM was utilized to semi-quantitatively identify the health risk associated with fungal and bacterial surface contamination within the hospital. Monitoring for allergens can help characterize environments with respect to specific allergens (e.g., fungi and/or bacteria). Both fungi and bacteria secrete enzymes that act as allergens. (Pope, Patterson et al. 1993) Source or reservoir samples have been used as indicators of exposure to indoor allergens and measurement interpretations can be semi-quantitative (e.g., "presence or absence" or "low, medium, or high). (Pope, Patterson et al. 1993) Environmental bacteria also grow in all wet spaces and are found in most cases where there is mold growth. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004)

The American Industrial Hygiene Association's consensus document *A Strategy for Assessing and Managing Occupational Exposures* (Mulhausen and Damiano 1998) served as the basis for the HRM. The HRM utilized criteria and recommendations of the Centers for Disease Control and Prevention (CDC 2003), US Environmental Protection Agency (USEPA 2001), American Conference of Governmental Industrial Hygienists (ACGIH 1999), Institute of Medicine (Pope, Patterson et al. 1993), the New York City Department of Health and Mental Hygiene (NYCDHMH 2006), the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE 2003) and the Vanderbilt University Medical Center (VUMC 2006) in establishing the risk factors for the model. A literature search was conducted to determine if the organisms identified via surface sampling within the Hospital were allergenic, pathogenic or opportunistic, and capable of producing fungal or bacterial toxin. The HRM resulted in a Health Risk classification of the space controlled by each AHU.

Health Risk was classified as High, Medium, Low, and de Minimis. The risk classifications were determined with input from experts in medical microbiology, industrial hygiene, public health, engineering controls, infection control, and medicine. A de Minimis risk score means that no indoor environmental contamination was found. A low risk score means the environmental conditions present do not indicate extensive biological contamination and/or the risk associated with adverse health affects to building occupants is low. A medium risk score indicates that environmental conditions present an increased risk for adverse health effects to building occupants due to environmental contamination and remediation is necessary. A high risk score indicates that conditions exist for adverse health effects due to exposure to biological contaminants and immediate intervention is necessary. Figure 9 below displays the HRM scores for the indoor space controlled by each AHU.

Indoor surface fungal and bacterial surface contamination was identified in every area of the hospital investigated. Air sampling confirmed the presence of indicators of indoor contamination in each of the spaces investigated. See **Section 4. Sampling Interpretation Summary** above. The spaces under the control of every AHU placed within the medium risk category. The environmental conditions are present such that immunocompromised or allergic patients are not fully protected against the risk of NI due to environmental bioaerosols. (Perdelli, Christina et al. 2006) Healthy hospital workers are not protected

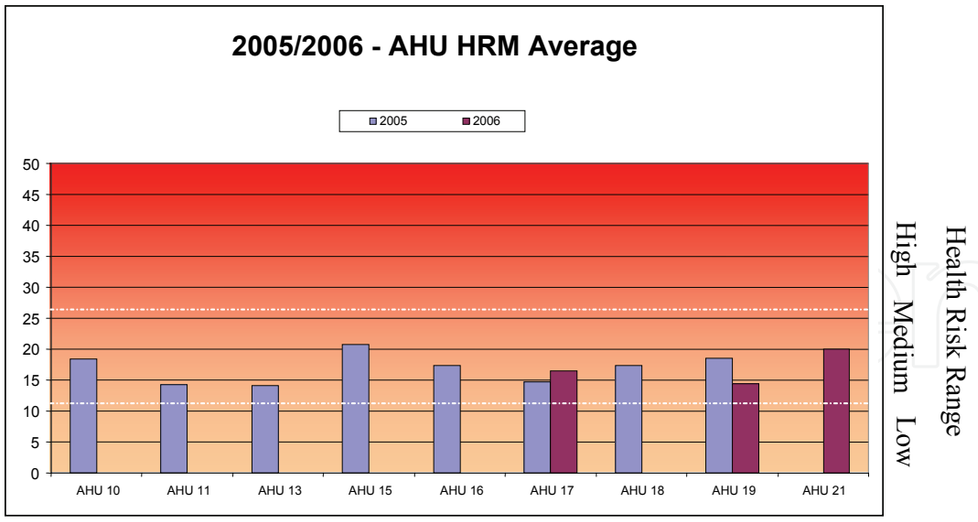


Fig. 9. Health Risk Model Scores for the space controlled by each AHU.

against allergic reactions to indoor bioaerosols growing within the facility and are at an increased risk of respiratory infections, including the common cold, sinusitis, tonsillitis, otitis, and bronchitis. (Institute of Medicine Committee on Damp Indoor Spaces and Health 2004) Hospital workers who are immunocompromised (e.g., diabetics, asthmatics, those undergoing cancer therapy or who have recent invasive surgery) are more susceptible to allergic reactions and the risk of work-related infections. The results of the HRM indicate that patients and staff are being exposed to microorganisms that are actively growing within the hospital which present a risk higher than what is expected in a hospital without water damage, microbial contamination, moisture infiltration, and OSA infiltration.

Periods of maintenance and non-routine operation of HVAC systems within the hospital can result in filter bypass, dissemination of biological contamination, and the infiltration of unfiltered OSA into the hospital, placing the hospital within the High Risk category due the creation of an exposure pathway during these times. Hence, times during and immediately after maintenance and non-routine operation of the HVAC systems present a high risk for health effects due to bioaerosols in the indoor environment. (CDC 2003)

6. Discussion and hypothesis testing

Indoor microbial contaminants and infectious agents are closely related to water and moisture-related conditions. (Bartley 2000) The scenario that has emerged within the hospital is one in which immunosuppressed or allergic patients are not fully protected against the risk of NI or allergies due to environmental bioaerosols. (Perdelli, Christina et al. 2006) Where an indoor environment is exhibiting growth or airborne suspension of bioaerosols, risk may be determined to exist even if levels are less than outdoor baseline and control levels. (Boss and Day 2003) While indoor bioaerosol concentrations were consistently lower than OSA, the biodiversity was consistently different. Air sampling indicators of an indoor source and microbial surface contamination were identified in each of the areas investigated.

The objectives of environmental control in buildings are to prevent or minimize occupant exposures that can be deleterious to human health and to provide for the comfort and well-

being of the occupants. (Pope, Patterson et al. 1993) The inability of the hospital's environmental systems in the areas investigated to manage moisture has resulted in a situation where patients, visitors, and staff are exposed to airborne microorganisms from indoor surfaces and OSA infiltration that normally would not be present within the building. This has resulted in an indoor environment that is not hygienic from the perspective of environmental contamination associated with the building and building systems.

Infection prevention in the hospital environment is one of the goals of healthcare workers and facilities. (Boss and Day 2003) Visitors, volunteers, and staff can both be infected and infectious. Highly filtered air is essential to preventing infection in healthcare facilities. (Boss and Day 2003) With the exception of AHU 11, all areas investigated were under the environmental control of an air conditioning system with >90% efficiency filters. The presence of the high efficiency final filters was largely responsible for the relatively low concentrations of total bioaerosols identified in the hospital ISA. Of particular concern is that a breach in the integrity of the final filters or dissemination of contamination during maintenance activities or non-routine operation could place the areas of the hospital which now fall into the medium risk category into the high risk category by creating an exposure pathway from contamination within the air handlers, OSA, and re-circulated air of the hospital to the occupant. The high efficiency filters within the AHUs are the hospital's main defense against nosocomial infection associated with indoor environmental contamination.

Although HVAC systems equipped with high efficiency filters can significantly reduce indoor concentrations of bioaerosols (Perdelli, Christina et al. 2006), the areas of the hospital investigated were under negative pressure, allowing the infiltration of unfiltered OSA, and had visible and confirmed microbial contamination on indoor surfaces and within each AHU. The presence of unfiltered air entering the Hospital via infiltration due to negative building pressurization is of concern (CDC 2003) and is significant risk factor for NI. (Streifel, Lauer et al. 1983; Rhame, Streifel et al. 1984; Rhame 1991; Nolard 1994) Biofilms (communities of bacteria in a matrix) and standing water were present in each of the AHUs investigated in 2005 and indicate the presence of excess moisture and poorly draining drain pans. Biofilms are conglomerates of microorganisms and indicate microbiological amplification within the AHUs. Visible surface contamination was identified within all the areas investigated.

Note that a breach in filter media or during times of HVAC system maintenance such as changing filters or starting and stopping the units, will cause an increase in airborne microorganisms or infiltration of unwanted OSA into the ISA of the Hospital. A failure or malfunction of any HVAC system component may subject patients to discomfort and exposure to airborne contaminants. Accumulation of dust and moisture in HVAC systems increases the risk for spread of health-care-associated environmental fungi and bacteria. If moisture is present in the HVAC system, periods of stagnation should be avoided. Bursts of organisms can be released upon system start-up, increasing the risk of airborne infection. If the ventilation system is out of service, rendering indoor air stagnant, sufficient time must be allowed to clean the air and re-establish the appropriate number of air changes once the HVAC system begins to function again. Reactivation of HVAC systems after shutdown can dislodge substantial amounts of dust and create a transient airborne increase of fungal spores. (CDC 2003) Therefore, during and after non-routine operation of the HVAC systems, the hospital is at high risk for nosocomial infection from environmental microorganisms.

The hypotheses testing results are:

Hypothesis A: The 90-95% final filters are controlling particulate matter generated by the AHUs and preventing contamination downstream of the filters. Note: AHU 11 does not have final filters.

Test: OSA versus ISA comparisons of total bioaerosols and spore traps.

Method: Investigator observations, interpretations, and literature review.

Test Result: Accept Hypothesis A—The final filters are controlling the dissemination of particulate matter and preventing the dissemination of particles downstream of the filters during routine operation. Statistical comparisons showed that indoor concentrations of non-culturable (spore traps) fungal bioaerosols were significantly lower than OSA concentrations. At least 79% (2005 data) of the non-culturable fungal bioaerosols are being removed by the final filters of the AHUs investigated, with the exception of AHU 11, which does not have final filters. At least 66% (2005 data) of the culturable fungal bioaerosols are being removed by the final filters of the AHUs investigated, with the exception of AHU 11, which does not have filters. With the exception of the bacterial (25°C) air samples in AHUs 10 and 11, the percent differences from outside air to indoor air were at least 35%. This is an indication that the filters were removing bacteria from both the outside and re-circulated air of the Hospital. The 2006 data showed a minimum percent reduction of 54% of all fungal samples (culturable and non-culturable) for the before filter to after filter comparisons. Therefore, particulate matter of similar aerodynamic diameters to fungi and bacteria are being removed from the air stream by the final filters.

Hypothesis B: The 90-95% final filters are preventing microbial contamination downstream of the filters.

Test: OSA versus ISA comparisons of bioaerosols.

Method: Investigator observations, interpretations, and literature review.

Test Result: Accept Hypothesis B—The final filters are preventing fungal particulate dissemination downstream of the filters by filtering the particles from the airstreams during routine operation. See Test Result for Hypothesis A. Indoor concentrations of non-culturable fungal bioaerosols were consistently lower and spore trap sampling results indicated that indoor concentrations of fungal bioaerosols were significantly lower than indoor concentrations. At least 66% (2005 data) of the culturable fungal bioaerosols are being removed by the final filters of the AHUs investigated, with the exception of AHU 11, which does not have filters. The 2006 data showed a minimum percent reduction of 54% of all fungal samples (culturable and non-culturable) for the before filter to after filter comparisons. The final filters are preventing the dissemination of bacterial contamination downstream of the filters during routine operation. Species of bacteria were identified within the AHUs and not identified post-filter, indicating that the filters were preventing the dissemination of contamination downstream. Indoor bacterial concentrations were consistently lower than outdoor concentrations. With the exception of the bacterial (25°C) air samples in AHUs 10 and 11, the percent differences from outside air to indoor air were at least 35% for the 2005 data. For the 2006 data, the minimum percent difference from OSA to ISA for bacteria was 64%. This is an indication that the filters were removing bacteria from the air streams. Note that indoor concentrations of bacteria are expected to be higher than outdoors but were consistently lower in the Hospital.

Hypothesis C: Staff is being exposed to potentially harmful concentrations of biological contaminants.
Test: OSA versus ISA comparisons of bioaerosols.
Method: Air samples, indoor surface sampling, HRM, Investigator observations, interpretations, and literature review.
Test Result: Accept Hypothesis D—Both the 2005 and 2006 data show indicators of indoor contamination and confirmed microbial surface contamination in each area investigated. Analysis for biodiversity showed that the bioaerosol profiles of ISA and OSA were consistently different. The HRM places all of the areas investigated in the medium risk category, indicating that staff are at risk for adverse health effects due to environmental contamination.
Hypothesis D: Patients are being exposed to harmful quantities of biological contaminants.
Test: OSA versus ISA comparisons of bioaerosols.
Method: Air samples, indoor surface sampling, HRM, Investigator observations, interpretations, and literature review.
Test Result: Accept Hypothesis E—Both the 2005 and 2006 data show indicators of indoor contamination and confirmed microbial surface contamination in each area investigated. Analysis for biodiversity showed that the bioaerosol profiles of ISA and OSA were consistently different, especially for species of *Penicillium* and *Aspergillus*. The HRM places all of the areas investigated in the medium risk category, indicating that patients are at risk for adverse health effects due to environmental contamination.

7. Conclusion

This 2-year investigation of bioaerosol concentrations in a hospital facility demonstrates that consideration of error, sampling variability, identification of microorganisms at the species level, and at least 6 replicate samples per location are necessary to detect significant differences in bioaerosol concentrations. Furthermore, spore trap samples were not sufficient to detect these differences regardless of the number of samples taken. Environmental investigators must consider this in their investigation strategy. Of particular importance is the failure of spore trap sampling to detect the same differences in airborne fungal bioaerosol species identified by culturable air sampling. Spore trap sampling should be considered a tool to determine a gross estimation of the quality of the indoor environment with regards to fungal bioaerosols and should not be used to make estimations on health or bioaerosol exposure. In all cases, spore trap sampling failed to detect differences in the aerobiological profile, masking significant concentrations of airborne fungal bioaerosols, including species of *Aspergillus* and *Penicillium*.
Statistical validity, analytical/sampling error, and species diversity should be considered for any bioaerol sampling intended to make comparisons on airborne concentrations of bioaersols between two or more environments. Identification of surface contamination should be considered de facto evidence of exposure to potentially harmful biological compounds. This study shows that high efficiency filtration can result in the environmental control of airborne bioaerosols. This is especially important in the hospital setting. PCR sampling shows promise because longer air sampling times can be used, surface samples can detect the presence of organisms on surfaces for an indication of a building's microbial burden, and because of relatively simple PCR sampling methods.

8. References

ACGIH (1999). Bioaerosols: Assessment and Control. Cincinnati, OH, American Conference of Governmental Industrial Hygienists.

ACOEM (2002). Adverse Human Health Effects Associated with Molds in the Indoor Environment, American College of Occupational and Environmental Medicine: 1-10.

AerotechP&K. (2006). "Microbial and IAQ Glossaries." Retrieved August, 2006, from www.aerotechpk.com.

Agency, U. S. E. P. (2001). "Mold remediation in schools and commercial buildings." USEPA EPA 402-K-01-001.

Anzai, Y., Y. Kudo, et al. (1997). "The phylogeny of the genera Chryseomonas, Flavimonas, and Pseudomonas supports synonymy of these three genera." Int J Syst Bacteriol 47(2): 249-251.

Arnow, P. M., M. Sadigh, et al. (1991). "Endemic and epidemic aspergillosis associated with in-hospital replication of Aspergillus organisms." J Infect Dis 164(5): 998-1002.

ASHRAE (1992). Gravimetric and Dust-Spot Procedures for Testing Air-Cleaning Devices Used in General Ventilation for Removing Particulate Matter Atlanta, GA, American Society of Heating, Refrigerating, and Air-Conditioning Engineers: 32.

ASHRAE (1999). Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size Atlanta, GA, ASHRAE: 41.

ASHRAE (2003). 2003 ASHRAE handbook : heating, ventilating, and air-conditioning applications. Atlanta, Ga., ASHRAE.

Bartley, J. M. (2000). "APIC state-of-the-Art report: the role of infection control during construction in health care facilities." Am J Infect Control 28(2): 156-169.

Beggs, C. and K. Kerr (2000). "The Threat Posed by Airborne Micro-Organisms." Indoor Built Environmnet 9: 241-245.

Bendig, J. W., P. J. Mayes, et al. (1989). "Flavimonas oryzihabitans (Pseudomonas oryzihabitans; CDC group Ve-2): an emerging pathogen in peritonitis related to continuous ambulatory peritoneal dialysis?" J Clin Microbiol 27(1): 217-218.

Boss, M. J. and D. W. Day (2003). Biological risk engineering handbook : infection control and decontamination. Boca Raton, Fla., Lewis Pub.

Burge, H. A. (2000). The Fungi. Indoor Air Quality Handbook. J. D. Spengler, J. M. Samet and J. McCarthy, McGraw-Hill: 45.41-45.33.

Burton, D. J. (2006). Industrial Hygiene Workbook: the Occupational Health Sciences. Bountiful, UT, IVE, Inc.

CDC (1992). "Public health focus: surveillance, prevention, and control of nosocomial infections." Morb Mort Wkly Rep 42(42): 783-787.

CDC (2003). Guidelines for environmental infection control in health-care facilities: Recommendations of CDC and the HealthCare Infection Control Practices Advisory Committee. Atlanta, GA.

Conover, W. J. (1999). Practical nonparametric statistics. New York, Wiley.

Dillon, H. K., P. A. Heinsohn, et al. (2005). Field guide for the determination of biological contaminants in environmental samples. Fairfax, VA, American Industrial Hygiene Association.

Dillon, H. K., P. A. Heinsohn, et al. (1996). Field guide for the determination of biological contaminants in environmental samples. Fairfax, VA, American Industrial Hygiene Association.

Dulworth, S. and B. Pyenson (2004). "Healthcare-associated infections and length of hospital stay in the medicare population." American Journal of Medical Quality 19(3): 121-127.

Eickhoff, T. C. (1994). "Airborne nosocomial infection: a contemporary perspective." Infection Control and Hospital Epidemiology 15(10): 663-672.

Esteban, J., M. L. Valero-Moratalla, et al. (1993). "Infections due to Flavimonas oryzihabitans: case report and literature review." Eur J Clin Microbiol Infect Dis 12(10): 797-800.

Fox, B. C., L. Chamberlin, et al. (1990). "Heavy contamination of operating room air by Penicillium species: identification of the source and attempts at decontamination." Am J Infect Control 18(5): 300-306.

Freeman, J. and J. E. McGowan, Jr. (1978). "Risk factors for nosocomial infection." J Infect Dis 138(6): 811-819.

Freeman, J. and J. E. McGowan, Jr. (1981). "Differential risk of nosocomial infection." Am J Med 70(4): 915-918.

Freney, J., W. Hansen, et al. (1988). "Postoperative infant septicemia caused by Pseudomonas luteola (CDC group Ve-1) and Pseudomonas oryzihabitans (CDC group Ve-2)." J Clin Microbiol 26(6): 1241-1243.

Fung, F. and W. G. Hughson (2002). Health effects of indoor bioaerosol exposure. Indoor Air.

Fung, F. and W. G. Hughson (2003). "Health effects of indoor fungal bioaerosol exposure." Appl Occup Environ Hyg 18(7): 535-544.

Gaynes, R. and J. R. Edwards (2005). "Overview of nosocomial infections caused by gram-negative bacilli." Clin Infect Dis 41(6): 848-854.

Georgiev, V. S. (1998). Infectious diseases in immunocompromised hosts. Boca Raton, CRC Press.

Gibson, K. J. C., M. Gilleron, et al. (2003). "Structural and functional features of Rhodococcus ruber lipoarabinomannan." Microbiology 149: 1437-1445.

Hitchcock, P. J., M. Mair, et al. (2006). "Improving performance of HVAC systems to reduce exposure to aerosolized infectious agents in buildings; recommendations to reduce risks posed by biological attacks." Biosecur Bioterror 4(1): 41-54.

Hollis, D. G., F. W. Hickman, et al. (1981). "Tatumella ptyseos gen. nov., sp. nov., a member of the family Enterobacteriaceae found in clinical specimens." J Clin Microbiol 14(1): 79-88.

Institute of Medicine Committee on Damp Indoor Spaces and Health (2004). Damp indoor spaces and health. Washington, DC, National Academies Press.

Kalliokoski, P. (2003). "Risks caused by airborne microbes in hospitals - source control is important." Indoor and Built Environment 12: 41-46.

Kerr, J. R., J. E. Moore, et al. (1995). "Investigation of a nosocomial outbreak of Pseudomonas aeruginosa pneumonia in an intensive care unit by random amplification of polymorphic DNA assay." Journal of Hospital Infection 30: 125-131.

Kiris, S., U. Over, et al. (1997). "Disseminated Flavimonas oryzihabitans infection in a diabetic patient who presented with suspected multiple splenic abscesses." Clin Infect Dis 25(2): 324-325.

Lai, C. C., L. J. Teng, et al. (2004). "Clinical and microbiological characteristics of Rhizobium radiobacter infections." Clin Infect Dis 38(1): 149-153.

Lalucat, J., A. Bennasar, et al. (2006). "Biology of Pseudomonas stutzeri." Microbiol Mol Biol Rev 70(2): 510-547.

Lam, S., H. D. Isenberg, et al. (1994). "Community-acquired soft-tissue infections caused by Flavimonas oryzihabitans." Clin Infect Dis 18(5): 808-809.

Lentino, J. R., M. A. Rosenkranz, et al. (1982). "Nosocomial aspergillosis: a retrospective review of airborne disease secondary to road construction and contaminated air conditioners." Am J Epidemiol 116(3): 430-437.

Lin, R. D., P. R. Hsueh, et al. (1997). "Flavimonas oryzihabitans bacteremia: clinical features and microbiological characteristics of isolates." Clin Infect Dis 24(5): 867-873.

Liu, P. Y., Z. Y. Shi, et al. (1996). "Epidemiological typing of Flavimonas oryzihabitans by PCR and pulsed-field gel electrophoresis." J Clin Microbiol 34(1): 68-70.

Lucas, K. G., T. E. Kiehn, et al. (1994). "Sepsis caused by Flavimonas oryzihabitans." Medicine (Baltimore) 73(4): 209-214.

Macher, J. (1999). Bioaerosols: assessment and control. Cincinnati, Ohio, American Conference of Governmental Industrial Hygienists.

Marin, M., D. Garcia de Viedma, et al. (2000). "Infection of hickman catheter by Pseudomonas (formerly flavimonas) oryzihabitans traced to a synthetic bath sponge." J Clin Microbiol 38(12): 4577-4579.

McDonald, L. C., M. Walker, et al. (1998). "Outbreak of Acinetobacter spp. bloodstream infections in a nursery associated with contaminated aerosols and air conditioners." Pediatr Infect Dis J 17(8): 716-722.

McNeil, M. M. and J. M. Brown (1994). "The medically important aerobic actinomycetes: epidemiology and microbiology." Clinical Microbiology Reviews: 357-417.

Montgomery, D. C. (2001). Design and analysis of experiments, John Wiley and Sons, Inc.

Mulhausen, J. R. and J. Damiano (1998). A strategy for assessing and managing occupational exposures. Fairfax, VA, AIHA Press.

Munro, R., G. Buckland, et al. (1990). "Flavimonas oryzihabitans infection of a surgical wound." Pathology 22(4): 230-231.

Nolard, N. (1994). "[Links between risks of aspergillosis and environmental contamination. Review of the literature]." Pathol Biol (Paris) 42(7): 706-710.

NYCDHMH (2006). "Guidelines on Assessment and Remediation of Fungi in Indoor Environments." The New York City Department of Health and Mental Hygiene.

Page, E. H. and D. B. Trout (2001). "The role of *Stachybotrys* mycotoxins in building related illness." AIHA J 62: 644-648.

Palleroni, N. J., M. Doudoroff, et al. (1970). "Taxonomy of the aerobic pseudomonads: the properties of the Pseudomonas stutzeri group." J Gen Microbiol 60(2): 215-231.

Perdelli, F., M. L. Christina, et al. (2006). "Fungal contamination in hospital environments." Infection Control and Hospital Epidemiology 27(1): 44-47.

Podbielski, A., R. Mertens, et al. (1990). "Flavimonas oryzihabitans septicaemia in a T-cell leukaemic child: a case report and review of the literature." J Infect 20(2): 135-141.

Pope, A. M., R. Patterson, et al. (1993). Indoor allergens: assessing and controlling adverse health effects. Washington, D.C., National Academy Press.

Pope, A. M., Patterson, R., Burge, H., Ed. (1993). Indoor Allergens. Assessing and controlling adverse health effects. Washington, D.C., National Academy Press.

Prezant, B., D. M. Weekes, et al., Eds. (2008). Recognition, Evaluation, and Control of Indoor Mold, American Industrial Hygiene Association.

Rahav, G., A. Simhon, et al. (1995). "Infections with Chryseomonas luteola (CDC group Ve-1) and flavimonas oryzihabitans (CDC group Ve-2)." Medicine (Baltimore) 74(2): 83-88.

Redd, S. C. (2002). "State of the Science on Molds and Human Health." Center for Disease Control and Prevention, U.S. Department of Health and Human Services.: 1-11.

Reina, J., J. Odgardd, et al. (1990). "Flavimonas oryzihabitans (formerly CDC group Ve-2) bacteremia in a pediatric patient on assisted ventilation." Eur J Clin Microbiol Infect Dis 9(10): 786-788.

Reisler, R. B. and H. Blumberg (1999). "Community-acquired Pseudomonas stutzeri vertebral osteomyelitis in a previously healthy patient: case report and review." Clin Infect Dis 29(3): 667-669.

Rhame, F. S. (1991). "Prevention of nosocomial aspergillosis." J Hosp Infect 18 Suppl A: 466-472.

Rhame, F. S., A. J. Streifel, et al. (1984). "Extrinsic risk factors for pneumonia in the patient at high risk of infection." Am J Med 76(5A): 42-52.

Romanyk, J., R. Gonzalez-Palacios, et al. (1995). "A new case of bacteraemia due to Flavimonas oryzihabitans." J Hosp Infect 29(3): 236-237.

Smith, G. E. (1990). Sampling and identifying allergenic pollens and molds. San Antonio, TX, Blewstone Press.

Spicer, R. C. and H. J. Gangloff (2000). "Limitations in Application of Spearman's Rank Correlation to Bioaerosol Sampling Data." AIHA J 61(May/June): 362-366.

Stetzenbach, L. D., M. P. Buttner, et al. (2004). "Detection and enumeration of airborne biocontaminants." Curr Opin Biotechnol 15(3): 170-174.

Stetzenbach, L. D. and M. V. Yates (2003). The dictionary of environmental microbiology. San Diego, Calif., Academic Press.

Straus, D. C., Ed. (2004). Sick Building Syndrome. Advances in Applied Microbiology. London, Elsevier Academic Press.

Streifel, A. J., J. L. Lauer, et al. (1983). "Aspergillus fumigatus and other thermotolerant fungi generated by hospital building demolition." Appl Environ Microbiol 46(2): 375-378.

Taneja, N., S. K. Meharwal, et al. (2004). "Significance and characterisation of pseudomonads from urinary tract specimens." J Commun Dis 36(1): 27-34.

USEPA (2001). Mold remediation in schools and commercial buildings. United States Environmental Protection Agency. EPA 402-K-01-001.

VUMC (2006). Infection Control Interventions During Construction in Patient Care Areas. Procedure 10-10.17. Nashville, TN, Vanderbilt University Medical Center. 10-10.17.

Walsh, T. J. and A. H. Groll (1999). "Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century." Transpl Infect Dis 1(4): 247-261.

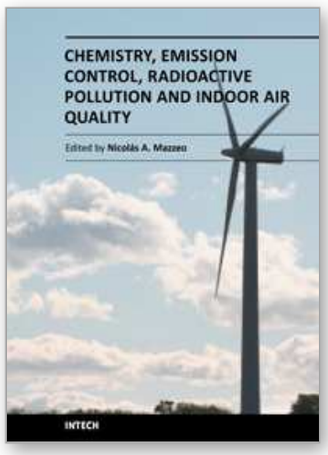
Weber, A. and E. Page (2001). "Renovation of contaminated building materials at a facility serving pediatric cancer outpatients." Applied Occupational and Environmental Hygiene 16(1): 2-31.

Wenzel, R. P. (1997). Prevention and control of nosocomial infections. Baltimore, William & Wilkins.

Wilson, M. (2005). Microbial inhabitants of humans : their ecology and role in health and disease. New York, Cambridge University Press.

Wilson, S. C., W. H. Holder, et al. (2004). "Identification, remediation, and monitoring processes used in a mold-contaminated high school." Adv Appl Microbiol 55: 409-423.

Yee-Guardino, S., L. Danziger-Isakov, et al. (2006). "Nosocomially acquired Pseudomonas stutzeri brain abscess in a child: case report and review." Infect Control Hosp Epidemiol 27(6): 630-632.



Chemistry, Emission Control, Radioactive Pollution and Indoor Air Quality

Edited by Dr. Nicolas Mazzeo

ISBN 978-953-307-316-3
Hard cover, 680 pages
Publisher InTech
Published online 27, July, 2011
Published in print edition July, 2011

The atmosphere may be our most precious resource. Accordingly, the balance between its use and protection is a high priority for our civilization. While many of us would consider air pollution to be an issue that the modern world has resolved to a greater extent, it still appears to have considerable influence on the global environment. In many countries with ambitious economic growth targets the acceptable levels of air pollution have been transgressed. Serious respiratory disease related problems have been identified with both indoor and outdoor pollution throughout the world. The 25 chapters of this book deal with several air pollution issues grouped into the following sections: a) air pollution chemistry; b) air pollutant emission control; c) radioactive pollution and d) indoor air quality.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen