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Descriptive analysis of airborne levels of Aspergillus fumigatus present in high-risk critical care patient areas; an eleven-year (2010 – 2021) surveillance study.

George H. Pineda
Georgia State University

Descriptive analysis of airborne levels of *Aspergillus fumigatus* present in high-risk critical care patient areas; an eleven-year (2010 – 2021) surveillance study.

By

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ABSTRACT

INTRODUCTION: As part of a microbial surveillance effort conducted over an eleven-year period, 38,765 culture samples were collected from various indoor and outdoor spaces within a children's hospital to determine the baseline levels of a fungus called *Aspergillus fumigatus*. *A. fumigatus* is an opportunistic fungus that has proven to be a highly dangerous microorganism; it is ubiquitous in the environment, it is an opportunistic and potent human pathogen and has a high mortality rate in hospitalized immune-compromised patients. In 2014, *A. fumigatus* was the leading cause of invasive mold infections in the United States in hospitalized patients causing 15,000 cases at the cost of $1.2 billion. Conducting surveillance of this organism in high-risk patient areas, such as intensive care units and operating rooms, is, therefore, a vital component of an effective invasive aspergillosis prevention program.

AIM: The study aimed to determine the level of *A. fumigatus* present in select areas of a children's hospital and determine the organism's baseline or "normal" level during periods of no outbreaks. The areas selected to be studied included high-risk areas where patients receive care and treatment and are therefore susceptible to infection; they include: (1) the outdoor environment; (2) intensive care units [ICUs] such as neonatal, cardiac, and pediatric; and (3) operating rooms [ORs], operating theatres or surgical suites. If baseline levels cannot be determined, the results can be compared to

published threshold limits to determine if the levels observed in the hospital pose a risk of invasive aspergillosis infection.

METHODS: The hospital provided for evaluation an electronic file consisting of a Microsoft Excel (Excel) spreadsheet containing microbial surveillance data in the form of total viable count (TVC). The TVC data was collected from viable samples obtained throughout the hospital property, explicitly looking for culture results for *A. fumigatus*. Sample results were reported as Colony Forming Units (CFUs) and collected from outdoor and indoor locations throughout the hospital. The study focused on identifying the locations within the hospital that posed the highest risk of infection by this organism; outdoor, intensive, and critical care areas, and operating room theatres. Next, culture results from each area were segregated using Excel by sorting all samples by location and omitting data if descriptions were incomplete. Finally, a descriptive statistical analysis was performed using the Excel data analysis tool pack to estimate various statistical parameters.

RESULTS: Of the 38,765 culture samples collected, 831 total viable counts (TVC) were positive for *A. fumigatus*. Of the total positives observed, the outdoor samples comprised 30.3% (252/831), the ICU comprised 9.7% (81/831), and the OR samples comprised 38.9% (323/831). Concerning the number of CFUs per sample, the outdoor sample contained a mean value of 8.3 CFUs, 95% CI (7.90 to 8.75), the ICU samples contained a mean value of 2.4 CFUs, 95% (1.77 to 3.02), and the OR samples contained a mean value of 3.1 CFUs, 95% CI (2.82 to 3.38). The outdoor sample result of 8.3 CFUs had an upper range of 75 CFUs, with a minimum of 1 CFU and a maximum of 76 CFUs. This high variability within the outdoor bioaerosol data is expected and a common observation likely influenced by spatial and temporal variations. The ICU result of 2.4 CFUs and the OR result of 3.1 CFUs contained a far smaller spread, as noted by the range of 30 and 36, respectively. In the case of

the ICU and OR, both high-risk areas exceed published threshold limits. The ICU result exceeds the "alert" threshold of 0.5 CFUs and the "action" threshold of 1.0 CFU. The OR result also exceeds the "alert" threshold of 0.1 CFU (with HEPA filtration), 0.6 CFU (without HEPA filtration), and the "action" threshold of 1.0 CFU.

DISCUSSION: Because ICUs and ORs are generally accepted as the clean patient care areas in the hospital, the ICU and OR are expected to contain the lowest concentrations of *A. fumigatus*. In the case of the OR, it is commonly accepted as the cleanest area of the hospital as it is engineered to be cleaned regularly, typically HEPA filtered, is positively pressurized, contains laminar flow, and undergoes significant air changes per hour. However, microbial surveillance data collected over an eleven (11) year period indicated that culture data within the ICUs and ORs were statistically higher than published threshold limits. When these thresholds are exceeded, the culture data should no longer be characterized as a normal or baseline level since the result is non-compliant and may present a significant risk of invasive aspergillosis. It is not known what the "safe" threshold level of *A. fumigatus* is, whereby there is no risk of invasive aspergillosis. However, it is clear that the presence of this organism in high-risk areas, such as the ICU and especially the OR, should be prevented and minimized to levels below 0.1 CFU.

Descriptive analysis of airborne levels of *Aspergillus fumigatus* present in high-risk critical care patient areas: an eleven-year (2010 – 2021) surveillance study.

By

George Pineda

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Approved:

Associate Professor: Lisa M. Casanova

Committee Chair

Research Associate Professor: Scott R. Weaver

Committee Member

May 2023

Date

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Author's Statement

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Chapter 1 – Introduction

Aspergillosis is an infection caused by a group of mold (fungus) of the genus *Aspergillus*. The origin of "Aspergillus" dates back to 1729 when an Italian priest and biologist named Pier Antonio Micheli looked through a microscope and noticed the unique shape and similarity to the Roman Catholic holy water sprinkler, which resembles the microscopic view of the fruiting structure of the Aspergillum (Walsh, 1989)[(2)].

Aspergillus includes almost 200 species, many of which are plant and human pathogens. They are present naturally in indoor and outdoor environments, and it is common for people to inhale mold spores, fruiting structures (conidiophores), and hyphal fragments daily without ever experiencing adverse health effects. A study that estimated the ambient background indoor or outdoor air concentrations for Aspergillus fumigatus (*A. fumigatus)* determined that we are constantly exposed to between 1 and 100 Colony Forming Units per cubic meter of air (CFU/m^3) throughout the year (Lacy, 1988)[(3)].

Filamentous fungal microorganisms from the genus Aspergillus are ubiquitous and saprophytic; i.e., they live and grow naturally in the environment and live on dead or decaying organic matter. However, they are also opportunistic pathogens that can be harmful to humans, especially those that are immuno-compromised and therefore have weakened immune systems (Mousavi, 2016)[(9)]. The main reservoir where *A. fumigatus* can be found in quantity is in areas containing abundant decaying vegetation, such as compost heaps, hay barns, and bird droppings. However, they can also be found in hospital environments, disturbed during construction/renovation projects (both indoor and outdoor disturbances), contaminated HVAC ventilation systems, and inadequate filtration (Nicolle, 2011)[(4)]. Hospital-acquired aspergillosis is usually associated with airborne fungal contamination of the hospital environment, especially during and after construction (Lemaire,

2018)[12]. This condition is often exacerbated when hospital ventilation systems draw in contaminated air from outside and indoors. Dust-containing microorganisms then settle within the insulated air plenum space and ducting of the HVAC system. Other common airborne fungal contamination sources include; a) non-filtered non-ventilated air, b) contaminated ventilation systems at intake or exhaust vents, and c) direct inoculation from contaminated material (Walsh, 1989)[2].

When exposed, some people may experience wheezing, coughing, shortness of breath, or an allergic response, such as allergic bronchopulmonary aspergillosis or ABPA. According to the Centers for Disease Control and Prevention (CDC), approximately 4.8 million people worldwide are affected by ABPA (Denning, 2013)[5]. The consequences of exposure can also be much more severe. About 1.67 million have chronic pulmonary aspergillosis (CPA), which often complicates pre-existing complications such as tuberculosis and sarcoidosis (Denning, 2011)[6] (Denning, 2013)[7]. *A. fumigatus* is the leading cause of invasive mold infections in people. *A. fumigatus* has also been shown to be a potent pathogen, as demonstrated by its high rate of mortality reaching up to 50% in hospitalized immunocompromised patients (Paulussen, 2016)[1] (Gonzalez-Garcia, 2022)[31]. Invasive aspergillosis (IA) can cause serious illness in people with weakened immune systems, such as individuals with existing lung disease, pre-existing health conditions, and immunocompromised persons. It is of particular concern in healthcare settings due to its ability to survive and grow even when exposed to antifungal drugs designed to kill them (CDC, 2019)[10]. IA occurs primarily in healthcare facilities where immunocompromised individuals undergo treatment and are hospitalized. In the United States, in 2014, nearly 15,000 people required IA hospitalization at an estimated cost of 1.2 billion (Benedict, 2019)[8]. Hospitalized patients can be classified according to the degree of risk of IA. In this context, the risk is determined from measures taken to protect at-risk patients from exposure to Aspergillus spores (Sub-Committee 2002)[16]. Measures include using High-Efficiency Particulate Air (HEPA) filtration, ventilation such as laminar flow, positive pressure, infection

control/aseptic techniques, and antifungal chemoprophylaxis (Humphries, 2004)[17]. The extent by which these control measures are provided is categorized as; (i) Group 1 – no evidence of risk, (ii) Group 2 – increased risk, (iii) Group 3 – high risk, and (iv) Group 4 – very high risk (See Table 1).

Table 1. Classification of At-Risk Patients*

Group #	Examples
Group 1 No Evidence of Risk	(1) Staff members, service providers, and contractors (2) All patients not listed in Group 2 – 4.
Group 2 Increased Risk	(1) Patients on prolonged courses of high-dose steroids, particularly those hospitalized for prolonged periods. (2) Severely immunosuppressed AIDS patients (3) Patients undergoing mechanical ventilation (4) Patients having chemotherapy who are not neutropenic (5) Dialysis patients
Group 3 High Risk "ICUs"	(1) Neutropenia for less than 14 days following chemotherapy (2) Adult acute lymphoblastic leukemia on high-dose steroid therapy (3) Solid organ transplantation (4) Chronic granulomatous disease of childhood (5) Neonates in intensive care units (ICU)
Group 4 Very High Risk "ORs"	(1) Allogenic bone marrow transplantation (2) Autologous bone marrow transplantation i.e., during the neutropenic period (3) Peripheral stem cell transplantation, i.e., during the neutropenic period (4) Non-myeloablative transplantation (5) Children with severe combined immune deficiency syndrome (SCIDS) (6) Prolonged neutropenia for greater than 14 days following chemotherapy or immunosuppressive therapy (7) Aplastic anemia patients

** Sub-Committee of the Scientific Advisory Committee. (2002). National guidelines for preventing nosocomial invasive aspergillosis during construction/renovation activities, Section 3. At-Risk Patients and Risk Factors, Page 15.*

According to CDC, aspergillosis is not a reportable infection in any U.S. state and is not nationally notifiable (Weber, 2009)[11]. Although it is not reportable, due to the pathogenicity and potential exposure to *A. fumigatus* in hospitalized immunocompromised patients, hospital infection control professionals have become very aware of the need to conduct surveillance and monitoring for the presence of this organism, especially in high-risk locations such as patient care areas (NICU, PICU, PACU), and very high-risk locations such as operating rooms theaters. As a result, healthcare facilities are left to develop their own surveillance methods to help identify potential sources of *A. fumigatus* and other pathogenic fungal and bacterial microorganisms. Microbial surveillance is an integral part of a hospital's infection control program: (1) it provides vital laboratory data regarding the types and counts of microbiota within the hospital, (2) it monitors the presence of specific nosocomial pathogens which may be the source of an outbreak, (3) it can determine the hospitals' baseline level, and (4) it allows comparison with published threshold limits for various organisms of interest. However, because routine monitoring and surveillance for Aspergillus is not required in hospitals, there are not many large data sets of Aspergillus occurrence in healthcare facilities. One large data set that does exist comes from a medium-sized U.S. children's hospital. During an eleven (11) year period, from 2010 to 2021, an undisclosed medium-sized U.S. children's hospital collected 38,765 culture samples at various indoor and outdoor locations as part of their microbial surveillance effort to monitor fungal and bacterial airborne contaminants. *A. fumigatus* was selected as the focus of this study due to this organism's high mortality rate in hospitalized immune-compromised patients (Paulussen, 2016)[1] (González-García, 2022)[31]. Therefore, the study's objectives were to determine the average total viable count per sample as measured in CFUs for three (3) high-risk locations observed within the hospital, compare these results to reference samples, and determine if the results could present an increased risk of IA. If there is a risk of IA, then suggest possible actions that can be implemented to reduce the risk.

Chapter 2 – Literature Review

A literature review was performed looking for published studies whereby organisms commonly associated with aspergillosis, particularly *A. fumigatus*, are observed in high-risk patient treatment and recovery areas where immuno-compromised patients may be exposed. The literature review focused on finding a baseline (commonly called "normal" or background levels) and recommended threshold limits. However, most studies conducted in healthcare settings were conducted during construction activities. Hospitals should implement preventative measures and surveillance monitoring to control fungal outbreaks of *A. fumigatus* and to regularly measure the risk of invasive fungal infections during hospital construction activities. Unfortunately, since hospitals have no regulatory requirements compelling them to report their findings, hospitals are reluctant to publish surveillance data, especially when the monitoring and testing results are unfavorable.

For this reason, finding published baseline surveillance data for *A. fumigatus* collected from ICUs or ORs is limited. On the other hand, finding baseline surveillance data for the outdoor environment is plentiful. However, as expected for outdoor samples, there is much variability within the bioaerosol data, much of which can be influenced by factors such as spatial and temporal variations, as well as interplaying factors to include: meteorological, local vegetation sources, agricultural, composting, all of which affect fungal growth and sporulation (Anees-Hill, 2022)[13].

Finding studies where a threshold limit or recommended "cut-off" level is identified is also limited for the same reasons why there is limited baseline data; there are no regulatory requirements and the reluctance of hospitals to publish surveillance data.

Table 2. Literature Review of Aspergillus sp. and A. fumigatus

Author, Year	Organism	Type	Location	Concentration
ISO 14698 [25]	Bacteria/Fungi	Threshold	OR	0.1 CFU/m^3
	Bacteria/Fungi	Threshold	ICU	5.0 CFU/m^3
Rhame, 1984 [26]	A. fumigatus	Baseline	Outdoor	6.3 CFU/m^3
Nicolle, 2011 [4]	Aspergillus sp.	Baseline	Outdoor	0.6 – 21.1 CFU/m^3
Arnow, 1991 [21]	A. fumigatus	Threshold	OR	1 CFU/m^3
O'Gorman, 2011 [18]	Aspergillus sp.	Threshold	OR	0.5 CFU/m^3
	Aspergillus sp.	Threshold	OR	0.16 – 0.40 CFU/m^3
NDSC, 2002 [16]	Aspergillus sp.	Threshold	OR	0.1 CFU/m^3
	Total Fungal Count	Threshold	OR	1.0 CFU/m^3
Morris, 2000 [27]	Aspergillus sp.	Threshold	OR	0.1 CFU/m^3
	Aspergillus sp.	Threshold	ICU	5.0 CFU/m^3
EU, 2008 [28]	Total Fungal Count	Threshold	OR	< 1 CFU/m^3
Aboul-Nasr, 2014 [19]	A. fumigatus	Surveillance	ICU	0.03 – 1.35 CFU/m^3
	A. fumigatus	Surveillance	OR	0.06 – 0.27 CFU/m^3
Aboul-Nasr, 2014 [19]	Aspergillus sp.	Surveillance	ICU	0.97 – 19.93 CFU/m^3
	Aspergillus sp.	Surveillance	OR	2.22 – 5.37 CFU/m^3
Sherertz, 1987 [24]	Aspergillus sp.	Surveillance	OR	0.009 CFU/m^3
Warris, 2001 [22]	Aspergillus sp.	Threshold	OR	0.1 CFU/m^3
Vonberg, 2006 [20]	Aspergillus sp.	Threshold	OR	0 CFU/m^3
Talento, 2019 [29]	Aspergillus sp.	Threshold	OR	<1 CFU/m^3
	Aspergillus sp.	Threshold	ICU	<5 CFU/m^3
Sajjadi, 2018 [30]	Aerobic Count	Threshold	OR	<1 CFU/m^3

Chapter 3 – Methodology

3.1 – Sampling Methods

The hospital's surveillance data was provided as a Microsoft Excel spreadsheet containing; i) a unique tracking code, ii) who collected the sample, iii) the date of sample collection, iv) the sample location, and v) the laboratory result. The laboratory results were provided as the Total Viable Count

(TVC) for each organism identified and reported in Colony Forming Units (CFUs). Due to the description of the sample location and conditions under which the samples were collected, all samples are assumed to be air samples. However, the results were considered semi-quantitative or presence/absence because no sample air volumes were provided. Therefore, they were reported as CFUs and not a quantitative result which must be reported as organisms per unit of air, usually expressed as CFU/m^3.

Microsoft Excel (excel) was used for data cleaning and interpretation. Descriptive statistics were then generated using the data analysis tool pack to summarize the data set. When the description of sample locations did not accurately and definitively categorize the sample data into specific groups based on location, the data was not included in the analysis. However, once the data set was segregated and classified, the observed culture data were compared to available reference samples.

The locations selected in this study included; (1) the outdoor environment, (2) intensive care units or ICUs, and (3) operating rooms (ORs). The outdoor location was selected as a sample for comparison. The intensive care unit (ICU) and operating room (OR) were selected due to their highest risk of infection. ICUs are considered high-risk specialty department within a hospital that provides care for patients experiencing potentially life-threatening conditions due to a critical injury or illness. ICUs are cleaned periodically and typically require nurses and doctors to wear scrubs to reduce the risk of infection. On the other hand, ORs are considered the highest-risk patient care area in the hospital as this is where operations are carried out in an aseptic environment. ORs are considered cleanrooms and are cleaned regularly before and after each surgical case and are terminally cleaned after the last case of the day. ORs are positively pressurized and require a high air volume to create and maintain sufficient air changes per hour and laminar flow. The HVAC system is usually HEPA filtered, although not always.

Table 3. Description of Patient Areas

Outdoor	High Risk	Includes all areas outside ("outdoor) of the hospital building. Includes rooftop areas, balconies, entryways into the hospital, and HVAC intake (be mindful of proximity to vegetation and moisture/water, exposure to UV, traffic, etc.)
ICUs	High Risk	Cardiac care unit (CICU or CITU), Geriatric care unit (GICU), Isolation (IICU), Medical (MICU), Long-Term (LTICU), Neonatal (NICU), Neurologic (Neuro-ICU), Pediatric (PICU), Psychiatric (PICU), Surgical (SICU), Trauma (TICU).
ORs	Highest Risk	General, Orthopedic, Neuro, Spine, Urology, Cardiac, Thoracic

The culture data from the three high-risk areas are then compared to a reference level (i.e., expected or threshold). The baseline levels refer to the average "normal" concentration of *A. fumigatus* that the hospital would expect to find during periods of no outbreaks. Expected levels represent the data set based on contingency tables, theory, published studies, and historical and objective data. Threshold levels are typically regulatory limits, guidelines, or recommendations gathered from previous studies. Based on their findings, they have been recommended as a threshold or cut-off bioaerosol level in specific hospital environments where patients may be at a high risk of IA. Unfortunately, no current regulatory or consensus-based strict numerical limits for Aspergillus counts accurately assess whether a particular location within a hospital is contaminated. Several studies have recommended a threshold limit that can be used to provide a warning of a possible outbreak of aspergillosis. For this study, two (2) threshold limits have been provided; (1) alert and (2) action. A threshold "alert" is a value that, if exceeded, should initiate the surveillance monitoring team members, such as infection control nurses, physicians, and industrial hygienists, to monitor the area carefully, possibly limit the use of the patient care area until culture counts can be lowered, conduct more frequent testing of the air and possibly

surfaces, and inspect the HVAC system. The threshold "action" is a value that, if exceeded, should immediately cause the shut-down and isolation of the HVAC system supplying the patient areas, conducting frequent air and possibly surface sampling.

While there is no consensus in the healthcare industry regarding having a clear cut-off concentration, permissible exposure limit, or threshold value that would designate a patient care area as being safe, unsafe, or immune to the risk of acquiring IA, there have been several studies that have recommended their not-to-exceed threshold. Many of these thresholds are identified in Table 2 and were based on the incidence of acquiring an infection, either from organisms from the genus Aspergillus or from the species *A. fumigatus*.

A descriptive statistical analysis was performed within each location, looking for the total viable count of *A. fumigatus*. Comparing these observations with published studies showing microbial surveillance data for *A. fumigatus* in other hospitals in similar conditions suggests that this invasive pathogen is present at the substantially higher background and recommended threshold limits.

This suggests that the hospital's original surveillance monitoring data set, initially thought to be a baseline measurement to determine and establish the presence of a "normal" level of *A. fumigatus* in the hospital, was more likely an indication that a potential outbreak of *A. fumigatus* was occurring. Not having an established threshold limit for comparison likely prevented the infection control team from being notified and recognizing that an aspergillus outbreak may occur.

Monitoring for *A. fumigatus* can be performed using qualitative or quantitative methods, and sampling can be performed using active or passive methods. There is uncertainty about the methods used to collect each sample for this study and missing information in the data set. Usually, a sampling plan for this type of data would include; (1) chain-of-custody, (2) sampling methods used, (3) laboratory reports, (4) calibration data, (5) media used, (6) equipment used, (7) volume or area sampled, (8) laboratory used for the analysis, (9) qualification of professional collecting the samples.

Due to the description of the sample location and conditions under which the samples were collected, all samples were considered air samples. However, a chain-of-custody (COC), certificate of laboratory results, and other support information were not provided to allow confirmation that all samples were air samples. If so, they were probably active air samples collected on agar plates using impact samplers, a standard method for sampling of this type. The most widely used method for bioaerosol sampling to collect active air samples is impact samplers. Active impact air sampling typically collects 1,000 liters of air (1 m^3) to achieve a detection limit of 1 CFU/m^3.

For this reason, it is common to collect 1,000 liters of air and report the results in CFUs as the total viable count (TVC). If passive air sampling is performed instead of active air sampling, the resulting surveillance monitoring data can be used as both methods are comparable (Napoli, 2012)[(14)]. There is no evidence that samples were collected using passive settle plates or surface swabs. The surveillance data indicated that most samples were collected by the same industrial hygiene firm using accepted sampling methods.

The semi-quantitative surveillance data provided by the hospital were recorded and input into an Excel spreadsheet containing short and abbreviated sample information. TVCs were reported as CFUs from the hospital's outdoor and indoor environment and were gathered for samples collected from three (3) locations; (1) outdoor environment, (2) high-risk areas such as intensive care units including; pediatric (PICU), cardiac (CICU) and neonatal intensive care units (NICU), and (3) very high-risk areas such as operating rooms (ORs). The three locations (outdoor, ICUs, and ORs) were chosen for this study.

Culture data that could not be characterized into specific well-described locations were placed into an "other" indoor hospital environment category.

3.2 – Statistical Analysis

The surveillance data for this study was provided as a Microsoft Excel (excel) spreadsheet. The following data cleaning and interpretation were performed: (1) The variable, "Location," was created from the "Source_text." This variable was created for samples collected either in the "OR," "ICU," or "OUTDOOR" (outside the hospital), (2) Using the data analysis tool pack, descriptive statistics were generated for the CFU counts for each of the three locations (OR, ICU, and OUTDOOR), and (3) A box plot was generated using the CFU counts by location. There were also data points that were omitted: (1) samples that were not positive for *A. fumigatus*, (2) samples that were positive for *A. fumigatus* but did not provide a numeric value for CFUs, and (3) samples that were not able to be accurately sorted by location, as the location stated from "Source_text" variable was ambiguous.

Table 4. At-Risk Areas Included in the Study

Outdoor	
N/A	Outside the hospital building (Roof, Parking Lot, Outside/Entrance to Building – *A. fumigatus* fungal spores are drawn into the hospital's interior mechanical conveyance system, where they are dispersed into the ICU and OR).
Restricted and Semi-Restricted Patient Treatment and/or Recovery Areas	
Group 3 High Risk	ED, Pre-OP, OR Corridor, PICU, PACU, SICU, MICU, CICU, Acute Patient Rm
Group 4 Very High Risk	Ortho OR, Cardiac OR, Transplant, Bone Marrow, Burn Unit

3.3 – Reference Sample

Using a numerical reference concentration, such as an expected or threshold, to determine whether or not a particular sample is above or below an observed value of interest is standard practice. Once a comparison is made, a descriptive statistical analysis can be performed to summarize the data in an organized manner by providing indications of error, accuracy, or confidence levels. Descriptive

statistics include frequency, central tendency, variation, and position measures. From this summary data, a decision can be made whether a problem likely exists. Reference samples include;

(i) Expected Levels

Expected levels represent the data set based on contingency tables, theory, published studies, and historical and objective data. For example, based on the review of numerous published studies, one referenced the average outdoor *A. fumigatus* levels outside a hospital in Michigan, USA (O'Gorman, 2011)[18] and found the outdoor levels to average 6.25 CFU/m^3. Another study conducted in outdoor locations around Dublin, Ireland, reported over one year that while the *A. fumigatus* conidia were generally present at levels below 10 CFU/m^3, levels as high as 400 CFU/m^3 were occasionally measured. Similar variations and ranges have been observed throughout high-risk patient areas.

(ii) Threshold Limits (Alert and Action)

There are no strict numerical limits for airborne Aspergillus counts that accurately assess whether a particular location within a hospital is contaminated. However, there are several studies that, based on their findings, have been recommended as a threshold or cut-off bioaerosol level in specific hospital environments where patients may be at a high risk of IA. Although many of these studies vary in threshold limits, several studies agree that observed levels above 1 Colony Forming Unit (CFU) will require intensive evaluation as these levels pose an increased risk in the incidence of IA (Aboul-Nasr, 2014)[19] (Vonberg, 2006)[20] (Arnow, 1991)[21]. For example, in a study conducted by Warris et al., the author describes that even levels below 1 CFU (0.2 CFU to 1 CFU) can pose a four-fold increase in the risk of IA. For this reason, Warris et al.

assessed a threshold limit of 0.5 CFU for protective isolation suites, 0.1 CFU for HEPA-filtered ORs, and 0.6 CFU for non-HEPA filtered ORs (Warris, 2001)[22] (Sub-Committee, 2002)[23].

Chapter 4 – Results

There were 38,765 culture samples collected outdoors and indoors within the hospital; 4,419 CFUs (11.4%) were confirmed positive for *A. fumigatus*. In addition, 831 CFUs that were collected from the outdoor, ICU, and OR (See Table 6) were also positive for *A fumigatus*: the outdoor samples comprised 30.3% (252/831), the ICU comprised 9.7% (81/831), and the OR samples comprised 38.9% (323/831) of all positive results. The remaining positive samples totaling 21.1% (175/831) were collected from areas other than the outdoor, ICU, and OR.

When analyzing the positive results based on the number of samples collected, the outdoor sample contained a mean value of 8.3 CFUs (2098/252), the ICU samples contained a mean value of 2.4 CFUs (194/81), and the OR samples contained a mean value of 3.1 CFUs (1001/323). The 95% confidence limits were as follows; (1) the outdoor sample was 8.3 CFUs, 95% CI [7.90 to 8.75], (2) the ICU sample was 2.4 CFUs, 95% [1.77 to 3.02], and (3) the OR samples was 3.1 CFUs, 95% CI [2.82 to 3.38] (See Table 5).

Table 5. Descriptive Statistics

Parameter	OR n=323	ICU n=81	Outside n=252
Mean	3.1	2.40	8.33
95% Confidence Limit (Lower)	2.81	1.77	7.90
95% Confidence Limit (Upper)	3.38	3.02	8.75
Standard Error	0.25	0.49	0.62
Median	1	1	5
Mode	1	1	1
Standard Deviation	4.55	4.39	9.86

Sample Variance	20.74	19.24	97.20
Range	36	30	75
Minimum	1	1	1
Maximum	37	31	76
Total CFUs	1001	194	2098

Table 6. Surveillance Data Summary

Dates Collected	05/20/2010 – 01/31/2021 (11 years, 4 months)
Total Samples Collected	**(N = 38,765)**
Outdoor	1,726 (4.4%)
ICU's (NICU, PICU, PACU)	1,621 (4.1%)
Operating Rooms (ORs)	10,657 (27.5%)
Total Viable Counts (CFUs)	**(N = 4,419)**
All Areas	4,419 (11.4%)
Outdoor	2,098 (47.5%)
ICU's (NICU, PICU, PACU)	194 (4.4%)
Operating Rooms (ORs)	1,001 (22.6%)
Total POSITIVE Number of Samples	**(N = 831)**
Outdoor	252 (30.3%)
ICU's (NICU, PICU, PACU)	81 (9.7%)
Operating Rooms (ORs)	323 (38.9%)
Ave CFUs Per POSITIVE Sample {CFUs/Sample}	**Ave. CFU**
Outdoor	**8.3**
ICU's (NICU, PICU, PACU)	**2.4**
Operating Rooms (ORs)	**3.1**

The outdoor sample had a mean value of 8.3 CFUs but also had a large spread with many outliers (See Figure 1). The upper range was 75 CFUs with a minimum of 1 CFU and a maximum of 76 CFUs. When comparing these observed results from published studies of outdoor bioaerosol data,

there is also a wide variability and range, as demonstrated by Nicole et al., of 0.6 CFU/m^3 to 21.1 CFU/m^3. As noted by Nicole et al. in their work, this wide variability in our data may be a common occurrence influenced by spatial and temporal variations.

The ICU result of 2.4 CFUs and the OR result of 3.1 CFUs contained a far smaller spread, as noted by the range of 30 and 36, respectively. In the case of the ICU and OR, both high-risk areas exceed published threshold limits. The ICU result exceeds the "alert" threshold of 0.5 CFUs and the "action" threshold of 1.0 CFU. The OR result also exceeds the "alert" threshold of 0.1 CFU (with HEPA filtration), 0.6 CFU (without HEPA filtration), and the "action" threshold of 1.0 CFU.

When comparing the average CFU per location to the threshold limits (Table 8), the concentration of *A. fumigatus* is greater than the published threshold limits of: (a) 0.1 CFU "alert" level for HEPA-filtered ORs (b) 0.5 CFU "alert" level for ICUs, (c) 0.6 CFU "alert" level for non-HEPA ORs, and (d) an "action" level of 1.0 CFU.

The average CFU per sample of 2.3 CFU is 4.6 times (x) higher ($p = <0.01$) than the threshold alert level of 0.5 CFU and 2.3x higher ($p = <0.01$) than the threshold action level of 1.0 CFU for areas within the ICU patient care areas (Table 9). Results also indicate that depending on whether or not the OR is HEPA filtered or non-HEPA filtered, the observed value of 3.1 CFU is 5.1x to 31x times higher ($p = <0.01$) than the threshold action level and 3.1x higher ($p = <0.01$) than the threshold action level of 1.0 CFU for ORs. This observation suggests that the CFU/sample concentration is significantly greater than the alert and action threshold levels, which may pose an increased risk of IA.

Figure 1. Box Plot of Average CFU by Location

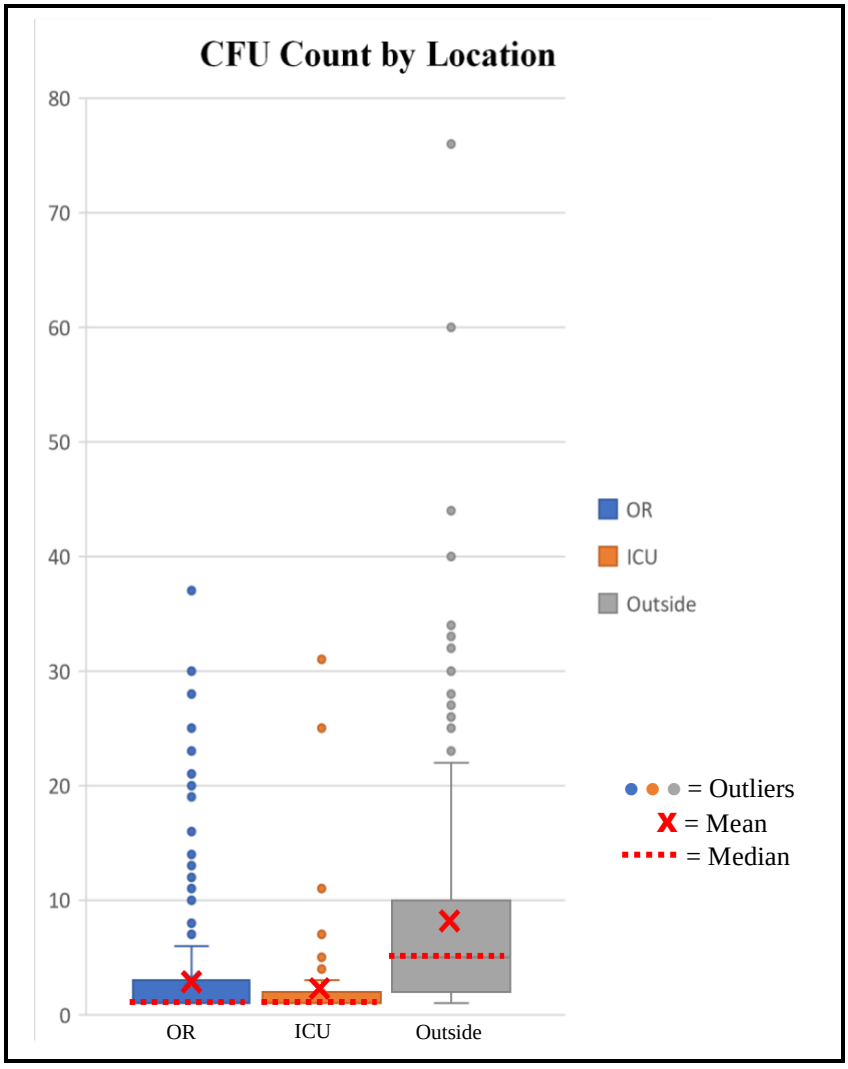
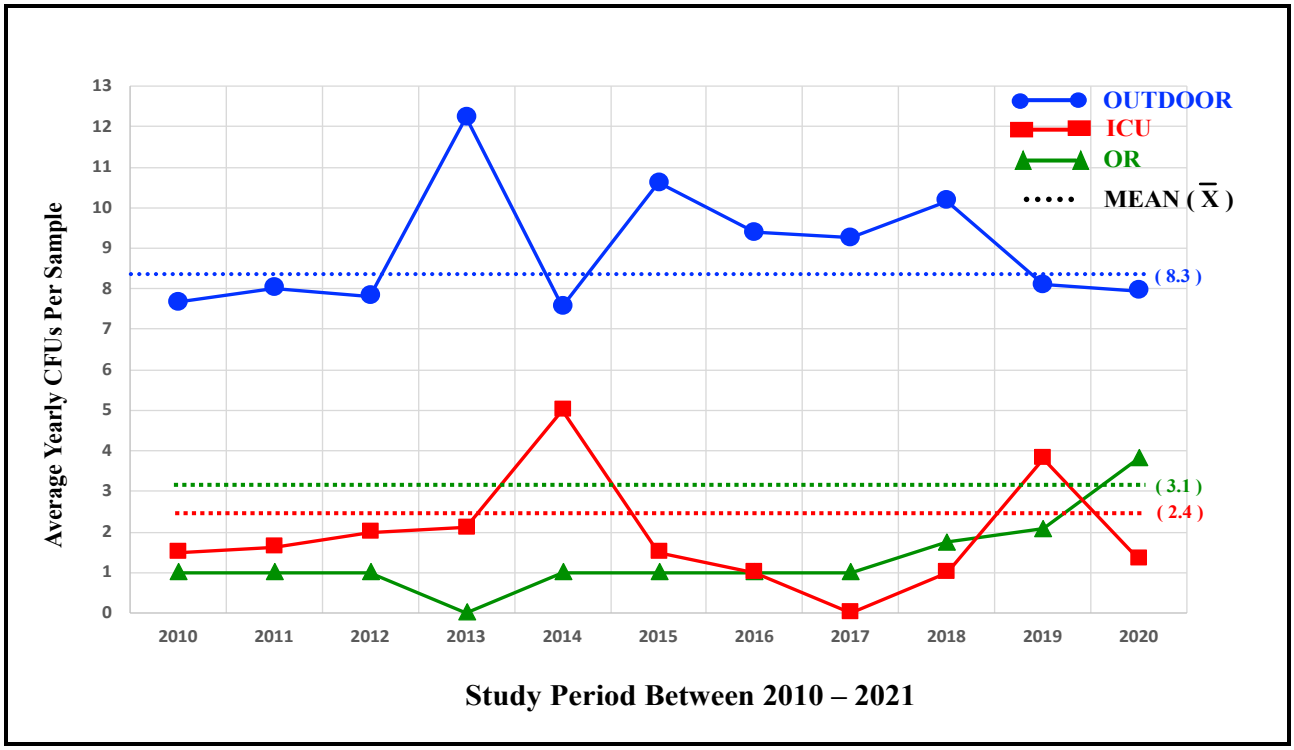


Figure 2. Counts Over Time



The study-wide CFU average for each patient area is provided in Table 5. However, when the data is further broken down on a year-by-year basis, one can see that there were fluctuations in the data. For example, as noted in Table 7 and Figure 2, between 05/06/2014 to 12/02/2014, the ICU recorded a spike of 5.0 CFUs per sample. Additionally, from 03/05/2019 to 12/10/2019, the ICU recorded a spike of 3.8 CFUs per sample. The OR also recorded spikes in the data as high as 2.1 CFUs between 03/05/2019 to 12/10/2019 and 3.8 CFUs between 01/09/20 – 12/29/20.

Table 7. – Spikes Observed During the Study

Area	Date of Spike	CFUs	Samples	CFUs/Sample	Location
ICU	05/06 – 12/02/14	40	8	5.0	NICU (30 CFUs on 12/06/14)
	03/05 – 12/10/19	61	16	3.8	NICU (25 CFUs on 08/20/19)
OR	01/30 – 12/20/19	192	92	2.1	ORs 7, 8, 9, 14
	01/09 – 12/29/20	807	211	3.8	OR 7

Table 8. – Comparison of Observed CFUs vs. Expected and Threshold Samples

CFUs/Sample Location	Observed (CFU)	Expected (CFU)	Threshold	
			Alert (CFU)	Action (CFU)
Outdoor	8.3	0.6 – 21.1**	---	---
ICU's (NICU, PICU, PACU)	2.4	0.50***	0.5	1.0
Operating Rooms (ORs) HEPA	3.1	0.009*	0.1	1.0
Operating Rooms (ORs) NON-HEPA		0.16 – 0.40*	0.6	1.0

*(Sherertz, 1987)[24]
**(Nicolle, 2011)[4]
***(Gorman, 2011)[18]

Table 9. – Statistical Analysis of Surveillance Data

CFUs/Sample Location	Threshold Alert (CFU)	Threshold Action (CFU)	Observed (CFU)	Significance
Outdoor	---	---	8.3	---
ICU's (NICU, PICU, PACU)	0.5	1.0	2.4	p = <0.01
Operating Rooms (ORs) HEPA	0.1	1.0	3.1	p = <0.01
Operating Rooms (ORs) NON-HEPA	0.6	1.0		p = <0.01

Chapter 5 – Discussion

The air sampling results obtained during the eleven-year study period showed that the average airborne concentration of *A. fumigatus* within the hospital's ICU and OR were elevated and exceeded the published threshold values used for this study. The study has also shown variations and spikes in the data, such as that observed for the ICU in 2014 and 2019 and the OR in 2019 and 2020. While the outdoor spikes and resulting spread in the data may be considered extreme in some cases, these concentrations are expected; they are consistent with published research studies and may be attributable to spatial and temporal variations common to the outdoor environment (Anees-Hill,

2022)[13]. Random spikes observed in the ICU and OR, however, are unexpected and may indicate that a microbial contamination problem exists. It is easier for an infection control and infection prevention (ICIP) team member to determine if a problem exists if there are established regulations, cut-off triggers, or threshold limit values that could be used to compare the results. The scope of this study, however, did not measure the incidence of disease or the mortality rate caused by *A. fumigatus* due to the exceedance of the threshold limits. Our study aimed to analyze the data, calculate the average airborne concentration of *A. fumigatus* present in high-risk patient areas such as the ICU and OR, and compare the results to published recommended limits and threshold values. In an established and sound surveillance program, the ICIP team should regularly test the air and surfaces and monitor and test the mechanical conveyance system (Sub-Committee, 2002)[16]. Any exceedance of microbial threshold limits or non-compliance with the mechanical conveyance system operation should alert the ICIP team to take evasive action, which may require a temporary shutdown of the space being serviced by the conveyance system.

In the future, the hospital's ICIP team should develop their own criteria limits or use the published threshold limits as we have done to determine whether or not a problem exists. If it is determined that threshold criteria limits have been exceeded, immediate action can be taken to investigate the source and cause of the possible microbial contamination. Depending on the investigation's findings, a decision can be made regarding the repair and restoration of the damaged item.

Developing a good surveillance program designed to regularly monitor and test the ICU and OR for the presence of *A. fumigatus* will require that hospital administrators support and are fully vested in the program (Liyanage, 2006)[32]. However, convincing hospital administrators to be proactive rather than reactive in the program may be difficult. Hospital administrators have in the past been reluctant to conduct surveillance and monitoring because there are no reporting requirements,

there are no regulations, there are no established criteria limits; federal agencies do not monitor the incidence of IA in healthcare settings, and should results ever indicate that a problem exists, this may be seen as an added liability to the hospital.

Traditional air and surface culture sampling as part of a good surveillance program is one of many ways to gather information regarding the possible contamination of *A. fumigatus*. Other methods can be used as surrogate testing to quickly alert the ICIP team of possible contamination or outbreak, including (1) DNA-tagged tracer particle analysis and (2) ISO cleanroom particle analysis. DNA-tagged tracer particle analyses are used to measure aerosols' transport through occupied spaces. These relatively new procedures (ref: veriDART, DNATrax, Poppy) will allow rapid analysis and evaluation using quantitative polymerase chain reaction (qPCR) assay for endpoint detection. Understanding the transport of aerosols will assist in determining the pathway of exposure through the ventilation system, which can often serve as a source of IA. These procedures may also help determine the efficacy of the HVAC ventilation and filtration system, which is used to mitigate the exposure risk.

Monitoring and compliance with International Organization for Standardization (ISO) cleanroom standards also provide a means to measure particle size and distribution in size ranges between 0.1 microns to >5.0 microns, which is well within the size of most airborne fungal spores and fragments. Monitoring is conducted using hand-held aerosol monitors, and results are compared to ISO threshold allowances depending on the class of cleanroom tested.

DNA-tagged tracer and ISO cleanroom particle analyses are alternatives to traditional air and surface surveillance monitoring. Whichever surveillance method is used for the hospital, there must be clear criteria limits with measurable endpoints to be an effective program.

Chapter 6 – Conclusions

The highest risk of IA in high-risk patient care areas such as the ICU and OR will occur when there is a breakdown, malfunction, or defect in the mechanical conveyance system, resulting in air contamination by fungal spores. If the fungal spores, such as *A. fumigatus*, happen to break the patients' sterile field during surgery, for example, and settle into the open wound, the organism may amplify and cause severe harm to the patient. This cycle can be interrupted by repairing any deficiencies within the conveyance system. However, to detect deficiencies within the ICU and OR, there must be regular surveillance and monitoring to rapidly alert the ICIP team, where they can alert the nurses and physicians of the discovery and prevent exposure from taking place. Therefore, relying upon systematic and regular air quality surveillance, using threshold limits, and rapidly repairing the identified damage will reduce the risk of IA.

LIMITATIONS

This study has several limitations, much of which originates from the uncertainty of the original electronic surveillance data provided by the hospital. The surveillance data is assumed to be accurate; however, no other supportive information was provided to confirm and validate the data. For example, in the case of presenting the data as a bioaerosol and airborne concentration reported as CFU/m^3, the researcher would need a chain-of-custody (COC) or a microbial laboratory report containing information such as; air sample volumes, flow rates, calibration, sample media, incubation temperatures, analytical methods, etc. This information should have been provided. Consequently, researchers are left to assume that individuals who collected the samples used standard and accepted practices commonly used in the industry. While we believe this was the case, it cannot be verified.

Another limitation was an accurate and detailed description of where each sample was collected. There were many instances where the vagueness in the description of the sample location prevented the classification of the sample result into the appropriate risk group.

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