Surveillance and Monitoring for Fungi During Construction

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Introduction

• Fungal spores present a risk of opportunistic infections
  – Both exogenous and endogenous sources

• Control is essential to the safety of immunocompromised patients
  – *Aspergillus* sp. represent greatest “exogenous” risk
Controlling the patient’s room

• Room pressurization

• Directional air flow

• Re-filtration or air cleaning
  – Address both endogenous and exogenous sources of contamination
• 53 outbreaks: 1967-2005
• 458 affected patients:
  – 299 (65.3%) haematological malignancies
  – Route of transmission: air
  – Site of primary infection: lower respiratory tract (356 patients)
  – Surgical site infections (24 patients)
  – Skin infections (24 patients)
Nosocomial aspergillosis

Unknown outbreak source; 12
Other source; 6
Air supply system; 9
Construction work (possibly); 3
Construction work (probably); 23
Species isolated

- A. fumigatus
- A. flavus
- A. terreus
- A. ustus
- Mixed
- Unknown
Ventilation as a source
Dust: a perfect home for *Aspergillus*!
Surveillance

• Healthcare associated aspergillus
  – Case
  – Antifungal drug consumption
  – Invasive fungal disease in targeted groups

• Air sampling

• Water sampling
LETTERS TO THE EDITOR

Routine sampling of air for fungi does not predict risk of invasive aspergillosis in immunocompromised patients

• 7-year sampling period: weekly: 978 samples
• *Aspergillus* spp. 16.7%: 1.8 cfu/m³ - 28.3 cfu/m³
• 45 cases proven IA (2.29% allo; 0.36% auto HSCT)
• cases of IA analysed 14 and 28-days following high counts
• Conclusion: high counts did not predict risk of developing IA

*Rupp et al. JHI 2008.*
Particle counting

- IQAir Particle Scan Pro
  Airborne Laser Counter
- $0.3\,\mu m - 5\,\mu m$
During demolition building was sealed and water sprayed to minimise dust emission

Particle and fungal concentrations monitored before and during demolition

Particle concentrations significantly higher during demolition

No difference in mould cultured at 37°C before and during demolition
Air quality monitoring of HEPA-filtered hospital rooms by particulate counting

Median particle counts of the patient rooms during a high risk period in 2005.

Air quality monitoring of HEPA-filtered hospital rooms by particulate counting

Particle counts of different locations

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean particle count (part/l)</th>
<th>Range</th>
<th>Number of measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 HEPA-filtered patient rooms of adult HSCT ward</td>
<td>174</td>
<td>7-6309</td>
<td>daily for 12 weeks</td>
</tr>
<tr>
<td>Intensive care unit (children), 3 patient rooms</td>
<td>5750</td>
<td>1370-21300</td>
<td>6 separate days</td>
</tr>
<tr>
<td>Regular adult patient ward - patient room - hallway</td>
<td>7450</td>
<td>3200-10600</td>
<td>hourly for one day</td>
</tr>
<tr>
<td></td>
<td>20870</td>
<td>12000-29000</td>
<td></td>
</tr>
<tr>
<td>Outside air</td>
<td>173659</td>
<td>110806-292624</td>
<td>6 separate days</td>
</tr>
</tbody>
</table>

Air sampler for quantitation of viable fungal spores

<table>
<thead>
<tr>
<th>Sampler type</th>
<th>Principle</th>
<th>Flow rate (litres/min)</th>
<th>Cut-off diameter (d50)(um)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sieve Impactor (Anderson)</td>
<td>Impaction on to agar plate</td>
<td>28.3</td>
<td>0.65–7.0</td>
</tr>
<tr>
<td>Slit sampler (e.g. Casella)</td>
<td>Impaction on to rotating agar plate</td>
<td>30–700</td>
<td>~0.5</td>
</tr>
<tr>
<td>Centrifugal Impactor (RCS)</td>
<td>Impaction due to centrifugal acceleration</td>
<td>40</td>
<td>4.0</td>
</tr>
<tr>
<td>Impingers (e.g. AGI)</td>
<td>Impingement into liquid</td>
<td>12.5</td>
<td>0.3</td>
</tr>
<tr>
<td>P.B.I. SAS Sampler (Single stage impaction)</td>
<td>Impaction on to agar plate</td>
<td>90/180</td>
<td>2.0</td>
</tr>
<tr>
<td>Settle plates</td>
<td>Gravity</td>
<td>Non-volumetric</td>
<td>N/A</td>
</tr>
<tr>
<td>Contact plates</td>
<td>Surface Sampling</td>
<td>Non-volumetric</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Air sampling: SAS Super 100 and Duo
Air sampler
Air sampling
Samplers: Andersen vs RCS

Table 1. Fungal genera most frequently isolated with the two air samplers.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Number of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Andersen sampler</td>
</tr>
<tr>
<td>Penicillium</td>
<td>35 (83)</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>33 (78)</td>
</tr>
<tr>
<td>Cladophialophora</td>
<td>31 (73)</td>
</tr>
<tr>
<td>Fusarium</td>
<td>21 (50)</td>
</tr>
<tr>
<td>Trichoderma</td>
<td>21 (50)</td>
</tr>
<tr>
<td>Rhodotorulla</td>
<td>15 (35)</td>
</tr>
<tr>
<td>Alternaria</td>
<td>15 (35)</td>
</tr>
<tr>
<td>Candida</td>
<td>–</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>–</td>
</tr>
<tr>
<td>Number of samples</td>
<td>42</td>
</tr>
</tbody>
</table>

RCS: Reuter centrifugal air sampler.

Figure 1. Correlation between fungal spore levels determined by measurement with the N-6 Andersen sampler and the Reuter centrifugal air sampler (RCS).

Brazilian Journal of Medical and Biological Research (2003) 36: 613-616
Indications for sampling

- To monitor levels of contamination prior to occupancy of special controlled environments e.g. to determine efficiency of HEPA filters in laminar flow facilities
- To identify potential sources of nosocomial aspergillosis when a case has been identified
- To predict environmental spore contamination from outside sources
- To identify defects/breakdown in hospital ventilation/filtration systems
- To correlate outbreaks of invasive aspergillosis with hospital construction or demolition work
- To monitor efficiency of procedures to contain hospital building wards where at-risk patients are managed
Method

• The air sample is aspirated through the instrument at a nominal rate of 180 litres/minute for a period of between 20 seconds and 6 minutes giving a volume range between 60 - 1080 litres

• The airflow is directed towards the agar surface of a 50 mm diameter contact plate that contains 12.5 ml of agar

• The plate is then removed for incubation
Location of sampling

• Choice of sampling height is 1.2 metres for room hygiene, with other samples taken for exploratory purposes near suspected or potential sources of contamination.

• Multiple samples are preferable to a single sample
  – For temporal and spatial variation in spore levels within any environment.
Sampling time

• Trial and error

• Not too long in sampling time in a heavily contaminated environment then the colonies
  – confluent growth - the colonies may even be uncountable
Laboratory procedure

• On receipt of the contact plates, these are placed in a pre-heated incubator to $28^0\text{C}$ for 5 days

• Identification of fungal colonies is based on colony characteristics and micro-morphological characteristics ascertained through microscopic examination at 400X magnification

• Specimens for examination should be prepared using a wet needle mount using lactophenol with cotton blue stain (0.75%)
Interpretation

• Levels of fungal spores vary by several orders of magnitude during the course of a day due to:
  – Activity levels in any one particular area
  – Fluctuations in temperature
  – Fluctuations in humidity
  – Fluctuations in air flow
  – Changes in light level
Monthly meteorological data for the period studied, including rain, mean temperature, wind speed and RH (%)
Seasonal pattern with peaks in summer

![Graph showing seasonal pattern with peaks in summer](image-url)
Interpretation

• Outdoor air (Note: seasonal variation recognised):
  – Total fungal count: $10^3$ to $10^5$ CFU/m$^3$,
  – Aspergillus: 0.2-3.5 conidia/m$^3$
• HEPA filtered air (>95% efficiency and >10 air changes per hour)
  – < 0.1 CFU/m$^3$
• No air filtration: 5.0 conidia/m$^3$
• Construction/defective ventilation: 2.3-5.9 conidia/m$^3$
• If total fungal count exceeds 1.0 CFU/m$^3$ on several occasions the air systems or procedural practice in patent areas requires intensive evaluation
Recommend to do further investigation of sources of contamination

• Total indoor counts > outdoor counts
• Comparison of indoor and outdoor levels of fungal organisms show one of the following:
  – Organisms are present in the indoor sample and not in the outdoor sample
  – The predominant organisms found in the indoor sample is different from the predominant organism in the outdoor sample
• A monoculture of an organism is found in the indoor sample. It may be absent from samples taken in other areas of the building
• Persistently high counts
Air sampling

• Targeted air sampling

• Written, defined, standardised, multidisciplinary protocol for sample collection and culturing

• Analysis and interpretation of results should use scientifically determined or anticipatory baseline values for comparison

• Expected actions, based on the results obtained, should also be defined

Chang CC. Internal Medicine Journal 44 (2014)
Recommended results analysis

- Best to look at performance trend and correlate with activities
- Exposure level of <5 CFU/m$^3$ of *Aspergillus* spp. in protective isolation areas
- <0.1 CFU/m$^3$ in HEPA-filtered environments, with limits of 15 CFU/m$^3$ for total colony counts of all fungal organisms


Further actions

• Start appropriate antifungal prophylaxis or pre-emptive therapy if not already used
• Perform an intensive retrospective review of microbiological, histopathological and post-mortem records for other cases
• Alert clinicians caring for high risk patients to the possibility of infection
• Establish a system for prospective surveillance of patients and their environment for additional cases
• If further cases arise in the absence of a nosocomial source consider monitoring home environments of patients pre-admission
Persistent high counts

- Sample:
  - dust
  - fabrics
  - ventilation ducts/screens/fans
  - ceiling voids
  - kitchen areas
  - excreta of roosting birds in close proximity of windows
Airborne *Aspergillus* contamination during hospital construction works: Efficacy of protective measures

Isabelle Fournel, MD, a Marc Sautour, PhD, b Ingrid Lafon, MD, c Nathalie Sixt, MD, b Coralie L'Ollivier, PhD, b Frédéric Dalle, PharmD, PhD, b Pascal Chavanet, MD, PhD, d Gérard Couillaud, MD, b Denis Caillot, MD, b Karine Astruc, MD, e Alain Bonnin, MD, PhD, f and Ludwik-Serge Ah-Go-Glé, MD g

Dijon, France

<table>
<thead>
<tr>
<th>Air treatment system</th>
<th>Before work</th>
<th></th>
<th></th>
<th>During work</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td></td>
<td>N</td>
<td>%</td>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>None</td>
<td>58/93</td>
<td>62.4</td>
<td></td>
<td>53/95</td>
<td>55.8</td>
<td></td>
<td></td>
<td>.36</td>
</tr>
<tr>
<td>HEPA filtration</td>
<td>0/134</td>
<td>0</td>
<td></td>
<td>2/234</td>
<td>0.8</td>
<td></td>
<td></td>
<td>.54</td>
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<tr>
<td>Plasmair</td>
<td>42/248</td>
<td>16.9</td>
<td></td>
<td>85/497</td>
<td>17.1</td>
<td></td>
<td></td>
<td>.95</td>
</tr>
<tr>
<td><em>Aspergillus</em> airborne</td>
<td>100/475</td>
<td>21.1</td>
<td></td>
<td>140/826</td>
<td>16.9</td>
<td></td>
<td></td>
<td>.07</td>
</tr>
</tbody>
</table>
The impact of portable high-efficiency particulate air filters on the incidence of invasive aspergillosis in a large acute tertiary-care hospital

Zakir-Hussain Abdul Salam, MBBS, MS, MPH, a Rubiyah Binte Harlin, BHSc, b Moi Lin Ling, MBBS, FRCPA, b and Kok Soong Yang, MBBS, MMedPH a

Singapore (Am J Infect Control 2010;38:e1-e7)

Table 1. Incidence rates and RRs of IA in different ward groups during the study period

<table>
<thead>
<tr>
<th>Ward group</th>
<th>Ward type</th>
<th>Incidence rate (per 1000 patient-days)</th>
<th>Period I (December 2005 to November 2006)</th>
<th>Period II (December 2006 to June 2008)</th>
<th>P value</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Wards with portable HEPA filters deployed December 2006</td>
<td>0.35</td>
<td>0.17</td>
<td>0.013</td>
<td>1.98</td>
<td>(1.11-3.51)</td>
</tr>
<tr>
<td>Group II</td>
<td>Wards with only fixed HEPA filters during the entire study period</td>
<td>0.16</td>
<td>0.31</td>
<td>0.061</td>
<td>0.51</td>
<td>(0.28-0.93)</td>
</tr>
<tr>
<td>Group III</td>
<td>Wards with no HEPA filtration</td>
<td>0.088</td>
<td>0.075</td>
<td>0.623</td>
<td>1.17</td>
<td>(0.44-3.10)</td>
</tr>
</tbody>
</table>
Outbreak lasted 2 years including 10 confirmed cases

Water was defined as the main source

Distribution of confirmed cases of fusariosis that occurred during an outbreak in a children’s cancer hospital

Clinical Microbiology and Infection, Volume 21 Number 3, March 2015
Environmental cultures performed during an outbreak of fusariosis in a children’s cancer hospital

<table>
<thead>
<tr>
<th>Room number</th>
<th>Cultures of the water</th>
<th>Cultures of swabs</th>
<th>Air cultures</th>
<th>Cultures of water after hyperchlorination</th>
<th>Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June 2009</td>
<td>August 2009</td>
<td>January to March 2010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shower</td>
<td>Tap</td>
<td>Drains and taps</td>
<td>Dry</td>
<td>Humid</td>
<td>Swabs</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>3</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>ND</td>
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<tr>
<td>4</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>5</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>10</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>–</td>
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<tr>
<td>11</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<td>12</td>
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<td>–</td>
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<tr>
<td>13</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
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<tr>
<td>14</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Isolation room 1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Isolation room 2</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

+, positive for *Fusarium*; –, negative for *Fusarium*; ND, not done; Humid, air collected during the flow of the shower in the adjacent bathroom; Dry, air collected before the shower was opened in the adjacent bathroom.

*For transplant patients.*
of the nosocomial water supply systems may serve as a route for systemic mould infection. Indeed, it has been demonstrated that fungal propagules may be aerosolised when contaminated water passes through shower heads, taps and toilet bowl, causing respiratory exposure in susceptible patients, especially in areas of major water use, such as showers. Those findings have supported the "wet route" of transmission for human systemic aspergillosis and fusariosis [4-6].

Despite the high incidence of mould infections, especially fusariosis and aspergillosis, in medical centres in Brazil and Latin America, there are few studies available addressing the presence of fungal pathogens in the water supply systems of medical centres in our region [7-9].

The occurrence of Fusarium infection in Paediatric Oncology patients undergoing Haematopoietic Stem Cell Transplantation (HSCT) prompted us to investigate the presence of microfungal contamination in the water distribution systems of the Oncology Paediatric Institute – GRAACC – UNIFESP, a tertiary care hospital devoted to the medical assistance of children with cancer.

Methods

Setting

The environmental surveillance of pathogenic fungi was conducted in the Oncology Paediatric Institute of the Federal University of São Paulo (UNIFESP), São Paulo, Brazil, a center with 300 new patients/year with a very busy day-hospital and 29 beds, including intensive care and HSCT units. The survey was conducted in the Paediatric Haematopoietic Stem Cell Transplant Recipient unit (HSCT), a division with four bedrooms and a total of six beds, all of which are equipped with a high efficiency particulate air filter (HEPA) and positive pressure (Figure 1).

Surveys

During a period of twelve months (March 2007-February 2008), we investigated monthly the water system supply of the HSCT unit by monitoring a total of fourteen different collection sites: the location at which the municipal water supply enters the hospital, four cold-water tanks (temperature about 25°C, two above-ground and two underground storage reservoirs) and nine sinks located in four bedrooms: four bathroom taps and one nurses’ station. These four cold-water tanks feeding all units included in this study. Before all water sampling, the target taps were flushed at maximum capacity for 5 minutes to rinse the accumulated dust and dirt from the pipes and tap. Samples from water reservoirs were collected using the Automatic Water Trap (Policontrol, São Paulo, Brazil). Next, each sample bottle was carefully sealed and immediately transported to the laboratory for further analysis. All samples were collected at environmental temperature.

Physicochemical analysis of water samples

Samples from water taps and tanks were collected every 30–40 days using sterile one-litre glass containers. All samples were collected at environmental temperature. The samples were transported and processed in the Special Laboratory of Mycology (LEMI), Federal University of São Paulo. Two different culture media were used to identify the different fungal species. Water physicochemical parameters were measured using a water quality meter (type 909, WTW, Singapore). The physicochemical analysis of samples was conducted in standard conditions.

Figure 1 Water distribution system facilities that were sampled during the environmental surveillance study of pathogenic fungi. This figure illustrates all collection sites: easel, tanks (T1 – T4), sink taps from 4 hospital rooms (B1–B4 represent suites) located on the same floor and a nurses station.

Mesquita-Rocha et al. BMC Infectious Diseases 2013, 13:289
• 1L samples from water taps and tanks were collected every 30–40 days using sterile one-litre glass containers
• Filtered and cultured on SDA plates for 15 days at 25°C and 37°C

Figure 2 Distribution of fungal propagules in water samples collected from 4 different seasons of the year.
Mould in tap water

• Free residual chorine rate varied from 0.14-0.89 mg/mL, with a mean of 0.38 mg/mL
  – Consistent with those established by the Brazilian Ministry of Health, ordinance no 518/2004, which set the standard for drinking water in Brazil
Water sampling

• High-risk patients avoid drinking tap water

• Targeted water sampling should be considered in comprehensive investigations of healthcare-associated fungal outbreaks
Conclusion

• Surveillance

• Monitoring
  – Sample as and when required
  – Follow up results over time
  – Use the service of a professional vendor

• Environment hygiene is one of core component of the IPC program
  – Air, water, general environment cleanliness (hygiene)
THANK YOU