Environmental surveillance and other control measures in the prevention of nosocomial fungal infections

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The steady world-wide increase in the number of severely immunocompromised patients in most hospitals has made the control and prevention of nosocomial systemic fungal infections a critical quality-of-care standard. Early diagnosis and antifungal prophylaxis of these infections are complicated, so avoiding the acquisition of the pathogen in the case of *Aspergillus* and minimizing the predisposing risk factors in the case of *Candida* are more effective approaches. The maintenance of good air quality in critical areas in hospitals is mandatory to reduce the incidence of invasive aspergillosis. We review the currently available Center for Disease Control recommendations and report our own experiences in the field. The indications and problems of fungal environmental and patient surveillance are also discussed.

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INTRODUCTION

In recent years there has been an inexorable increase in the number of highly immunocompromised patients in hospital environments [1–5]. Severe and prolonged neutropenia following chemotherapy is a major risk factor for systemic fungal infections [4,6,7]. However, the proportion of patients without neutropenia or severe immunosuppression also at risk of invasive fungal infections is higher than is usually thought [5,8–13].

Mortality associated with disseminated fungal infection remains elevated [5], probably owing to the difficulties of an early diagnosis. Clinical and laboratory diagnosis of these infections lacks sensitivity and specificity [5]. Several new approaches to prevention and diagnosis are being developed, such as the monitoring of *Aspergillus* antigenemia. Patients with presumed fungal infection require intense clinical and laboratory monitoring for signs of disseminated infection [4]. Early diagnosis may guide appropriate treatment and prevent mortality. However, decision analysis models are needed to minimize the number of diagnostic tests that are used to reach a final diagnosis in each group of at-risk patients. It is also necessary to establish whether to or not treat, given a set of clinical, radiological and laboratory results [14].

Prevention of nosocomial fungal infections is also problematic. Efforts may be addressed to prevent acquisition of the infection or treatment of the pathogen before it causes disease. In either case, implementation of preventive measures is costly, disruptive and involves diverse groups of hospital personnel. These experiences have led some experts to ask whether the prevention of these infections is even a realistic aim [15].

ETIOLOGY OF NOSOCOMIAL FUNGAL INFECTIONS

The rate of nosocomial fungal infections was 2.0 per 1000 discharges in USA in 1980 and 3.8 per 1000 discharges in 1990 [16]. It is estimated that this figure has risen since 1990, but there are no updated global reports.

The majority of nosocomial fungal infections (almost 80%) are caused by *Candida* spp. [16]. They are responsible for over 5% of all nosocomial infections [17]. *Candida* is the fourth nosocomial bloodstream micro-organism. Bloodstream infection with *Candida* this pathogen has the highest associated crude mortality (40–60%) [5,18].

Candida albicans ranks seventh among all hospital pathogens [5]. The fraction of infections caused by non-albicans *Candida* species is increasing; at present; almost 50% of bloodstream infections in surgical and neonatal intensive care units are caused by non-albicans *Candida* [18].

Aspergillus spp. is responsible for 1.3% of fungal nosocomial infections [16]. None the less, the incidence appears to be

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much higher in specialized care wards, such as in bone marrow transplant units [5].

Immunocompromised patients are highly susceptible to infections by organisms of 'low virulence', and newly recognized pathogens are continuously being reported, such as *Malassezia* spp., *Fusarium* spp., *Trichosporon* spp., *Mucor* spp., *Paecilomyces lilacinus, Pseudallescheria boydii, Scedosporium prolificans, Hansenula anomala* and *Blastoschizomyces capitatus* [19–27].

SOURCES OF INFECTION

The modes of transmission and portals of entry of fungal nosocomial infections vary according to the pathogen involved.

Candida is predominantly of endogenous origin but cross-infection via the hands of health-care workers or relatives or through different devices has been shown to occur [5]. *Aspergillus* causing clinical disease is usually acquired from outside, mainly through the respiratory tract or through direct inoculation (such as postoperative wounds, heart valves, etc.).

Acquisition of less frequent fungal infections may be endogenous, or related to hand carried or airborne fungi, or to previous tissue trauma, etc. In these infections, increased host susceptibility is thought to be the primary risk factor

PREVENTIVE MEASURES

Avoid acquisition from the environment

Air quality maintenance

The maintenance of good quality air in hospital contributes substantially to reduce the incidence of invasive aspergillosis. Aspergillus is ubiquitous in the external environment and numerous reservoirs have also been identified in hospitals: unfiltered air, ventilation systems, contaminated dust during hospital construction, carpeting, water food and ornamental plants [1,2,5,9,28].

Most patients with invasive aspergillosis (IA) present with pneumonia; therefore, it has been hypothesized that the inhalation of airborne spores, either directly or after intermediate nasopharyngeal colonization, is a direct cause of pulmonary infection in immunocommpromised patients [5].

The concentration of *Aspergillus* spores in the hospital air is the major extrinsic risk factor for the occurrence of nosocomial IA [2,5,28,29]. Therefore, the most obvious strategy to reduce IA is to diminish exposure of immunocompromised patients to *Aspergillus* conidia by using environmental control.

Environmental strategies are recommended for high-risk areas (bone marrow transplantation units, surgical rooms, etc.). Table 1 shows the Center for Disease Control (CDC) recommendations [28]. They include the use of the following:

(1) high-efficiency particulate air (HEPA) filtration of incoming air;
(2) directed room airflow;
(3) positive room-air pressure relative to the corridor;
(4) well-sealed rooms;
(5) high rates of room air changes.

The integrity of the air filtration system needs to be closely monitored with regular, planned preventive maintenance, spore counting, pressure monitoring and airflow changes [1,5,28]. In our experience, the practice of routinely undertaking air surveillance has been essential in the detection of failures in the system.

The use of HEPA filtration [10] and of laminar airflow [6,8] in high-risk units has been shown to reduce the risk of IA but does not reduce it to zero. The reasons for this apparent failure include the fact that some of the patients may already be colonized by *Aspergillus* when they enter hospital, or because patients leave protective environments to undergo diagnostic or therapeutic procedures [3,6]. Of course, a real leak in airflow must also be considered.

CDC guidelines recommend that hospital policies to minimize exposure of high-risk patients to potential sources of *Aspergillus*, such as hospital construction and renovation, cleaning activities, carpets, food, potted plants and flower arrangements, should be put into practice [28].

In facilities with no previous case of aspergillosis, dust accumulation should be prevented by daily damp-dusting horizontal surfaces and regularly cleaning ceiling and air-duct grills when the rooms are not occupied by patients [1,2,28]. Systematic review and coordination of infection control strategies with hospital personnel in charge of engineering, maintenance and catering should be undertaken.

When hospital construction and renovation activities are being planned, a strategy should be implemented to prevent patients at high risk of aspergillosis from exposure to high spore levels in the air. Impermeable barriers must be constructed between patient-care and construction areas to prevent dust from entering patient-care areas. Maintenance of a negative pressure in areas relative to adjacent patient-care areas is essential unless there are contraindications for such pressure differentials. Direction of pedestrian traffic away from con-struction areas prevents dust dispersion. Air and environmental monitoring for fungal spores may be indicated when building works adjacent to a high-dependency unit are taking place.

Owing to demolition work of a building close to our general hospital main building (Figure 1) this year we have demonstrated the validity and reliability of air sampling procedures before, during and after demolition (data not published). There was a clear elevation in the counts of

Table 1 Recommendations for the prevention and control of nosocomial pulmonary aspergillosis [28]

Recommendation	Category[a]
Staff education, especially care providers for immunocompromised patients	IA
Surveillance, with focus on	
High-risk patients (<1000 granulocytes/mm^3 for 14 days, <100 granulocytes/mm^3 for 7 days)	IB
Periodic review of microbiological, histopathological and postmortem data	IB
Periodic surveillance cultures of high-risk patients	Unresolved
New construction of specialized care unit for high-risk patients	
Minimization of fungal spore counts by HEPA filtration, directed airflow, positive pressure, proper seals, high rates of room-air changes	IB
Ultra-high air change rates (100–400/h), laminar airflow	Unresolved
Minimization of exposure of high-risk patients to construction and carpet and floor cleaning	IB
Prophylactic use of copper-8-quinolinolate biocide in fireproofing material	Unresolved
Existing facilities with no cases of nosocomial aspergillosis	
Minimize fungal spore counts as above	IB
Minimize exposures as above	IB
Conduct routine maintenance of the heating, ventilation, and air-conditioning system, including prevention of bird access to air intake ducts	IB
Minimize time and wear mask when high-risk patients are outside the area	IB
Eliminate potential *Aspergillus*-contaminated food, potted plants and flowers	II
During construction, erect barriers, direct pedestrian traffic away and clean new areas before entry to high-risk patients*	IB
After a case of nosocomial aspergillosis occurs	
Begin a retrospective review and prospective search of other cases	IB
If continuing infection occurs, conduct an environmental investigation	IB
Contact the local or state health department if assistance is needed	IB
Decrease host risk of infection	
Use of cytokines to decrease the duration of granulocytopenia	II
Intranasal amphotericin B or oral antifungal agents prophylactically	Unresolved

[a]Categories are as follows: IA, strongly recommended for all hospitals and supported by well-designed experimental or epidemiological evidence; IB, strongly recommended for all hospitals and viewed as effective by experts on the basis of strong rationale and suggestive evidence; II, suggested for implementation in many hospitals, supported by suggestive clinical or epidemiological studies with a strong theoretical rationale or definitive studies applicable to some but not all hospitals; Unresolved, practices for which insufficient evidence or consensus regarding efficacy exists.

filamentous fungal spores linked to demolition by controlled explosion (Figure 2). The sealing of doors and windows, the closing of external air ducts and the continuous air renovation and positive pressure in the operating theaters and in areas with a protective environment during demolition works proved efficacious in avoiding an increase in spore counts. No cases of IA were detected in hospitalized patients.

When a case of nosocomial *aspergillosis* occurs, the CDC recommends a prospective search for additional cases in hospitalized patients and an intensified retrospective review of the hospital's microbiology. Histopathology and postmortem records should also be investigated. If evidence of continuing *Aspergillus* infection exists, then an environmental investigation should be conducted to determine and eliminate the source.

Environmental surveillance is also recommended in the following situations: monthly surveillance in protected areas, during reformation works near high-risk areas, before patients enter a new protected area or after renovation and when there is suspicion of dysfunction in the quality of air systems.

For details of the practicalities of environmental surveillance [30], we refer the reader to Richardon's excellent article on the *Aspergillus* website (http://www.aspergillus.man.ac.uk). The most common method of measuring airborne conidial loads relies on an air filtration device. Other methods estimate the level of contamination by determining the number of conidia adhering to the walls of the patient's room by using contact methods with Petri dishes or swabbing with cotton-coated applicator sticks. Each sampling method has its merits and, at present, the method most frequently used in indoor air

Figure 1 Cloud of dust produced the day of the controlled demolition of our maternity hospital.

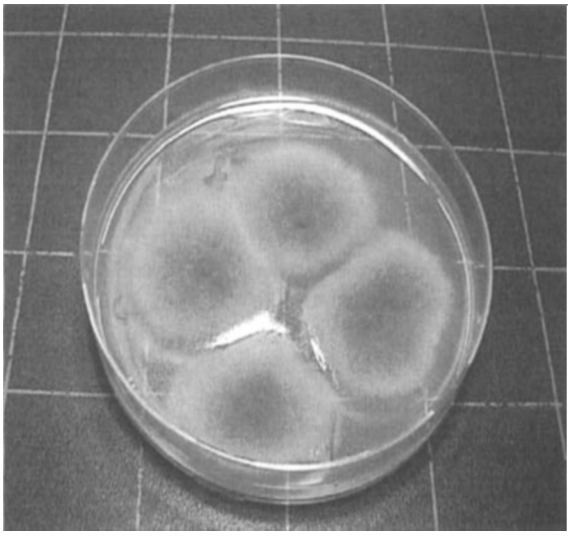

Figure 2 Agar plates with filamentous fungi obtained by sampling hospital air with an air filtration device.

surveys is impacting particles from an airstream onto an agar surface (Figure 3).

Unfiltered air averages 1–15 pathogenic *Aspergillus* spp. colony-forming units (CFU) per m^3, although short-term variations (such as seasonal variations) are substantial [31]. Although there is no reasonable estimation of a threshold of conidial concentration above which the risk of IA increases [4], most authors recommend *Aspergillus* air counts of less than 5 CFU/m^3 in the operating theater and in protective isolation suites, although counts of less than 0.1–1 CFU/m^3 are desirable. There is a great variability between countries (such as 10–200 CFU/m^3 according to the type of surgery).

The repeated isolation of the same fungal species from several samples or an increase in usual counts must raise concern about the existence of an environmental reservoir.

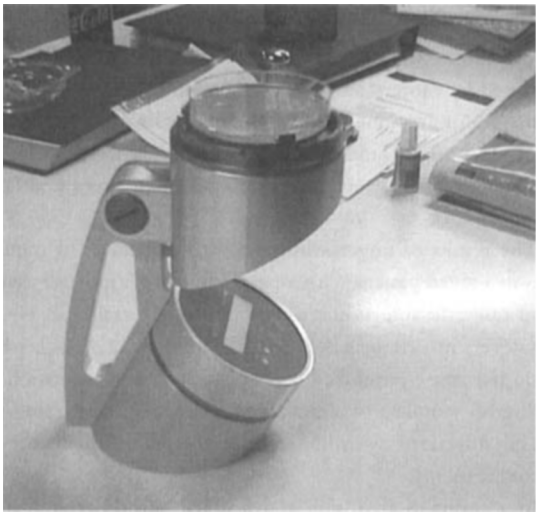

Fig 3 MAS-100 Air Sampler (Merck) and Sabouraud irradiated agar plates used in our hospital for sampling high-risk areas.

With regard to the operating theater, samples taken at the air-intake grill allow evaluation of the ventilation system. Samples taken at the center of the theater reflect the hygienic conditions of the room. It is common to find counts 2–3 times higher in these latter samples.

When the counts are over the acceptable limits and without a clear cause to justify them and to act on (such as works, ventilation system fault, etc.), the first action to take is to confirm the environmental sampling results. This is because any movement close to the sampling area, such as an air draught, may cause false-positive results. If the high count should persist, then the operating theatre or isolation ward will be closed. The hygienic condition of the airflow tubes and of the grills in the ventilation system must then be checked by the maintenance staff, as well as the correct technical performance of the system and the last change of filters.

Routine environmental sampling allows determination of reference air contamination limits to be used in every-day practice. With the routine use of the same method, its reliability and cost-effectiveness are guaranteed. However, the cost of investigating an outbreak of *Aspergillus flavus* in an operating room, just on environmental samples, may be high.

It is important to remember that genetic analysis cannot discriminate between clinical and environmental isolates of *A. fumigatus*, indicating that every strain present in the environment is a potential pathogen if it encounters the appropriate host [32]. Thus, the degree of exposure assessed by CFUs per unit of ambient air is not predictive of disease; the actual risk appears to vary with the underlying condition.

At present, only three methods can be used for genotypical typing of *A. fumigatus* strains [4]. Although most researchers have used the PCR- and RFLP-based typing methods

separately, studies are under way to compare their discriminatory potential and to evaluate if combination of data obtained by more than one typing method will lead to better strain discrimination [4,33]. To date, strain typing has been most successful by microsatellite polymorphism or analysis of Southern hybridization patterns obtained with repeated DNA sequences [4].

The results of fingerprinting clinical isolates from multiple sites in a given patient with aspergilloma or IA indicate that, in most cases, the infection is caused by a single strain [32,34–36]. However, in certain patients, tested under conditions which excluded the possibility of accidental contamination of biological samples, two strains were isolated, suggesting that mixed infections with different strains of *A. fumigatus* can occur in IA [4].

In a confined area such as a hospital, airborne conidia are extremely diverse, with 85% of the strains being isolated only once and each displaying a unique fingerprinting pattern [4]. The remaining 15% of the strains, which account for over 30% of the isolates, may each be isolated on several occasions, and may persist for several months in the same hospital environment [4]. This suggests that the majority of strains isolated are specific to each hospital and originate less commonly from the local outside environment.

If the isolation of the same fungal strain from the patient and the environment is the criterion used for 'hospital-acquired infection', then some 30–40% of the cases of IA are nosocomial [4,8,37]. However, even during outbreaks of aspergillosis, multiple patients are rarely infected by the same strain [34]. Each patient is surrounded by an extremely diverse population of strains. Furthermore, the absence of identity between genotypes found in patient-associated fungi and fungi found in their environment should not exclude a nosocomial origin of an infection [4]. The nosocomial nature of IA can be demonstrated even after several weeks of delay between acquisition of the fungus and the development of IA.

In summary, we believe that environmental sampling is a useful tool for determining the presence of a problem with the quality of the air in the hospital setting. However, its results must be interpreted with caution.

Water

Opportunistic fungal pathogens have been recovered from sinks and shower heads in several hospitals in the USA. *A. terreus* and *A. niger* were cultured from the shower heads, as well as *Fusarium* spp. Sampling before and after showering revealed a significant increase in spore counts in the air. The clinical significance of this finding remains unclear. *A. fumigatus*, which is by far the most frequent causative pathogen of IA, has not been recovered from water in any hospital in the USA, but it has been found in tapwater samples in a Norwegian hospital [38]. Some authorities feel that mould proliferation around sink outlets may represent another environmental reservoir, so water leaks should be cleaned up and repaired [2,8]. Nevertheless, the role of water in the transmission of aspergilli needs further elucidation.

Cross-transmission

Cross-transmission of filamentous fungi is very rare, although there are some reports. Fomites have been found to be the source of aspergilli in an outbreak of cutaneous aspergillosis [39]. Other sources identified include contaminated substance abuse material injected intravenously or contaminated cannabis [1].

Candida may be transmitted after contact with colonized health-care workers or patients' relatives (hands or oropharyngeal colonization). Meticulous handwashing or disinfection is the best preventive measure of cross-infection by *Candida*.

PROMPT DIAGNOSIS

Surveillance cultures

Fungal surveillance cultures have been studied as potential predictors of invasive or disseminated mycoses. Active surveillance of patients considered to be at high risk for fungal infection has enhanced case detection in some instances [37,40] and this is the main reason for its advisability.

Surveillance cultures of *Aspergillus* are indicated in a very specific set of patients. These include selected groups of transplant patients (solid organ transplant (SOT) and bone marrow transplant (BMT)), granulocytopenic patients (such as those with malignancies) and selected groups of HIV-positive patients [41]. The criteria used for indicating the performance of surveillance cultures are usually based on the presence of risk factors for invasive mycosis, such as the need for hemodyalisis after a solid organ transplantation or very low CD_4 counts in the case of HIV-infected patients.

Sputum cultures positive for *Aspergillus* have different sensitivities for the diagnosis of invasive respiratory aspergillosis. They range from 72% in patients with hematological malignancies, BMT or granulocytopenia, and 58% in SOT to 14% in HIV-positive patients [42,43]. Sensitivity also depends on the species isolated [43]. The sensitivity is as high as 98% if *Aspergillus fumigatus* is isolated from a respiratory sample in a heart transplant patient [43].

Sputum cultures also have different positive predictive values regarding the type of SOT. They range from 60% to 82%, 56%, 41–72%, 30–45% and 16% in bone marrow, heart, liver, kidney and lung transplants respectively [44]. These have also shown a good positive predictive value in leukemic patients during an outbreak [45]. Nevertheless, diagnosis of invasive disease using this method will be too late to aid in the clinical management of the patients.

Fungal surveillance cultures in BMT have shown that colonization is not necessarily predictive of fungal infection but may prompt more aggressive diagnosis or early treatment of potentially fatal invasive infections [29]. Routine nasal cultures have been recommended before transplantation, in cases of neutropenia longer than 7 days or in neutropenic patients with fever [45,46]. In some studies, samples taken from the tongue and perineum have shown colonization more often than those taken from the nostrils [29]. However, cultures from these sites are not of proven value as indicators of prophylaxis [47].

Regarding *Candida*, previous colonization is the most important independent risk factor for the development of invasive disease [5,40,48,49]. Very few patients have fungemia without a prior positive culture.

In several studies, fungal colonization of two or more noncontiguous anatomical sites correlates with an increase in the mortality risk of disseminated candidiasis (even higher than with fungemia) [48,50–52]. This is why, in every patient with clinically suspected candidiasis (symptomatic) or simply with multiple risk factors, it is advisable to obtain samples from the upper respiratory tract (pharynx and lung secretions), drains, surgical wounds, urine, gastric fluid, the skin around every intravascular catheter, blood and even feces (for a direct examination). It is important to detect this multiple colonization, because it usually precedes the onset of the systemic illness caused by *Candida* and, in some cases, more effective than blood cultures [53]. Detection of colonization by *Candida* of two or more noncontiguous anatomical sites in intensive care unit patients indicates the need for pre-emptive therapy [54].

Depending on the presence or absence of symptoms and infection risk factors, different courses of action were recommended by experts at a consensus conference [55]. In patients with symptomatic risk and even in those with no risk factors, they recommended surveillance cultures. The diagnostic value of these cultures in asymptomatic at-risk patients was not established. Considering the high attributable crude mortality (40%) of disseminated candidiasis in these patients, we believe that the possibility of colonization by *Candida* should be actively investigated, as we have already mentioned.

Apart from the number of different anatomical sites colonized, the type of *Candida* is also very important, e.g. it helps to determine the specific antifungal treatment and it also helps to find the origin of the infection. For instance, with regard to the origin, we know that *Candida parapsilosis* fungemia suggests infection of an intravascular device; so any catheters should be withdrawn. Isolation of *Candida tropicalis* has a high positive predictive value for disseminated disease, and it highlights a group of patients needing empirical treatment but not prophylaxis [49]. In some series, 80–100% of the patients colonized with *C. tropicalis* develops an invasive infection [56]. Obviously, surveillance cultures may no longer be necessary if prophylaxis is administered [57].

This is useful in the detection of existing patient colonization by any type of fungus, be it a yeast or a filamentous fungus. This comes in useful in the investigation of outbreaks, as occurred in a cluster of infections with *Blastoschizomyces capitatus* in the hematology ward in our hospital (unpublished data).

Serodiagnosis of invasive fungal infections

In contrast to immunocompetent hosts, growth of *A. fumigatus* in the tissues of immunodepressed hosts is not correlated with an increase in anti-*Aspergillus* antibody titers. In fact, the presence of anti-*Aspergillus* antibodies in immunocompromised individuals is more likely to represent antibody formed before the onset of the immunosuppressive therapy rather than the result of invasive infection [4]. Antibody detection in this population can be used prognostically but not diagnostically for IA.

In fact, the serological diagnosis of IA is based on the detection of circulating antigens in biological fluids (such as serum, urine and BAL) obtained from patients. For a detailed review on this matter, we refer the reader to two excellent articles by Latgé [4,14]. Briefly, the Platelia (Sanofi Diagnostic Pasteur) sandwich ELISA for the detection of galactomannan (GM) is currently the most sensitive method developed [58]. This test contributes to the early diagnosis of IA, even before signs and symptoms of the disease become apparent (median of 6 days). This is its most important feature, because the detection of antigenemia dictates the initiation of therapy. Another advantage is that the decrease in GM concentration in serum correlates with treatment efficacy.

The diagnosis of *Candida* by serology consists of the detection of mannan and antimannan antibodies [4,40]. The sensitivity of the test is good (85.7%), but the specificity and positive predictive value are low because of too many false-positive reactions (up to 50%).

The sensitivity of detection in serological tests must be improved. New tests under study include the detection of cell wall markers such as galactosaminoglycan or 1,3-d-glucan and an immuno-PCR method for GM.

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