Hospital sources of *Aspergillus* species: New routes of transmission?

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**Summary**

With the continuing increase in the number of severely immunocompromised patients, hospitals are faced with the growing problem of invasive aspergillosis and other opportunistic fungal infections. Since treatment of these infections are difficult and outcome is often fatal, preventive measures are of major importance in the control of invasive filamentous fungal infections. Until recently, inhalation of airborne conidia was believed to be the primary route of acquiring *Aspergillus* infection. Despite the fact, that efforts to filter the hospital air has led to a reduction of airborne conidia paralleled by a decrease in the frequency of invasive infections, the correlation between the concentration of *Aspergillus* conidia in hospital air and the risk of invasive infections remains unclear. Furthermore, alternative modes of transmission may exist and should be recognized and investigated. The discovery of hospital water as a potential source of *Aspergillus fumigatus* and other filamentous fungi may suggest a new route for the transmission of invasive filamentous fungal infections. Epidemiological studies, based on molecular characterization and comparisons of fungal isolates recovered from patients and environment, are needed to expand our understanding of these alternative routes of transmission.

**Key words**

*Aspergillus*, Environment, Transmission, Epidemiology

**Fuentes hospitalarias de Aspergillus spp.: ¿nuevas vías de transmisión?**

El aumento continuo del número de pacientes severamente inmunocomprometidos enfrenta a los hospitales con el creciente problema de la aspergilosis invasora y otras infecciones fúngicas oportunistas. Dado que el tratamiento de estas infecciones es difícil y, a menudo, el desenlace fatal, las medidas preventivas son de vital importancia en el control de las infecciones invasoras por hongos filamentosos. Hasta hace poco se consideraba que la vía principal de adquisición de la infección por *Aspergillus* era la inhalación de conidios aéreos. A pesar de que los esfuerzos por filtrar el aire de los hospitales han permitido una reducción importante de los conidios aéreos que ha ido acompañada de una disminución en la frecuencia de infecciones invasoras, sigue sin estar clara la correlación entre la concentración de conidios de *Aspergillus* en el aire del hospital y el riesgo de infecciones invasoras. Pueden existir formas de transmisión alternativas que deberían ser reconocidas e investigadas. El descubrimiento del agua del hospital como fuente potencial de *Aspergillus fumigatus* y otros hongos filamentosos puede sugerir una nueva vía de transmisión de infecciones invasoras por hongos filamentosos. Son necesarios estudios epidemiológicos, basados en la caracterización molecular y comparaciones de aislamientos fúngicos obtenidos de pacientes y del entorno para ampliar nuestro conocimiento de estas vías de transmisión alternativas.

**Palabras clave**

*Aspergillus*, Ambiente, Transmisión, Epidemiología

Opportunistic invasive fungal infections, in particular due to *Aspergillus fumigatus*, are an increasing problem in hospitalized, immunocompromised patients [1-3]. Patients at risk are those with hematologic malignancies [4,5], bone marrow or solid organ transplant recipients [6,7], patients with chronic granulomatous disease [8] and AIDS patients [9]. These patient groups have in common severe immunosuppression resulting from both granulocytopenia [10] and compromised cell-mediated immunity. The incidence as well as the mortality of invasive aspergillosis primarily depends on host factors and the timely
administration of effective therapy by early diagnosis. In transplant patients the incidence of invasive aspergillosis range from 0.7 up to 8.4% depending on the organ transplanted. Lung and bone marrow transplant recipients show the highest incidence numbers, whereas pancreas and kidney transplant patients have a lower risk of developing invasive aspergillosis [7,11]. The incidence of invasive aspergillosis in patients with acute leukemia, chronic granulomatous disease and AIDS are estimated at 5-24%, 25-40% and 0-12%, respectively [2]. Despite new approaches to therapy, mortality still ranges from 50% to 100% [2,7,12-16]. An overall success rate of 25% to 34%, varying substantially between different host groups, has been documented with amphotericin B (both conventional and lipid formulations), the currently standard therapeutic agent for the treatment of invasive aspergillosis [12,13,17-20]. It seems that better results are obtained in patients receiving itraconazole, although, this observation is not based on studies with direct comparison in well-described patient groups. The observation could be biased by the fact, that in general patients with less severe immunosuppression are likely to receive this new triazole antifungal with activity against Aspergillus spp. [21].

Therefore, prevention by the use of effective infection control measures is of high priority in the management of at-risk patients. The role of the hospital environment in the epidemiology and pathogenesis of invasive aspergillosis has been studied by many investigators. In this review, the current knowledge on the relation between these invasive infections and the presence of Aspergillus spp. in the hospital environment will be discussed.

The hospital environment

Air

Aspergillus species are ubiquitous organisms and can be found in every region in the world. Aspergillus spp. are commonly found in soil and decaying vegetation [22]. Air, however, plays a crucial role in the spread of Aspergillus spp. in the environment and in the transmission to patients. Aspergillus spp. efficiently release large amounts of conidia in the air from conidiophores that protrude from the mycelial mass. Due to their small size and roughened surface, Aspergillus conidia may remain airborne for prolonged periods. As conidia gradually settle out, anything in contact with air will become contaminated with conidia. The process of settling out and becoming airborne again can repeat itself for prolonged periods of time, since Aspergillus conidia are viable for months in dry locations.

Reported conidial concentrations of A. fumigatus in outdoor air vary from study to study [23-32]. Peak concentrations can be as high as 67 up to 185 colony-forming units per cubic meter (CFU/m³) [24,31,33]. Extremely high concentrations of airborne A. fumigatus conidia, up to 10⁶ conidia/m³, have been reported in special circumstances, such as near compost heaps and hay barns [40], or during demolition work [41]. Seasonal variation in conidial air counts has been documented. Highest concentrations of A. fumigatus have been detected either in summer [24], or during autumn and winter [26,34]. However, more recent studies suggest that the number of Aspergillus conidia in the air is not subjected to seasonal variation [33,35-39]. Nor could a correlation between airborne Aspergillus conidia and meteorological data, such as mean temperature, rainfall, air pressure or humidity be detected [24,27].

Several studies have shown large variability in the concentration of Aspergillus conidia in the air from sample to sample [27,29,35,36]. This may, at least in part, be due to sampling error. Sampling of air for Aspergillus is very problematic and single sample measurements of Aspergillus conidia are often not precise. Another reason for significant fluctuations in conidial counts between samples may be the occasional release of large amounts of conidia [29]. In a study, which performed continuous air sampling, only one burst of Aspergillus conidia was detected in the first 182 h of sampling [42]. As the frequency and nature of such bursts are insufficiently known, one may argue that sampling of small air volumes over short time periods results in unreliable estimates of the total exposure to Aspergillus conidia in individual patients. Recently, very useful recommendations regarding sampling of Aspergillus conidia in air were given in a review by Morris et al. [43]. Furthermore, in this review the various parameters influencing meaningful air sampling were outlined.

Recognition of the importance of the airborne route in the transmission of aspergillosis has lead to the recommendation and installation of high-efficiency particulate air (HEPA) filtration (alone or in combination with laminar air flow-LAF- systems) in bone marrow transplant and hematology units. Some studies indicate that HEPA-filtration without LAF is less effective in reducing conidial air counts than HEPA-filtration in combination with LAF [44,45]. Air filtration measurements have been shown to decrease the number of conidia in the air [25,30,32,44]. Furthermore, a gradient in the conidial air counts was found ranging from high levels in common sites to a virtual absence in rooms equipped with HEPA-filters [45]. A decreasing number of conidia was found when comparing outside- and inside- hospital air and when comparing open areas within in the hospital to the secluded department of hematology [37]. Most authors recommend Aspergillus air counts of less than 5 CFU/m³ in protective isolation suites, and less than 0.1 CFU/m³ in HEPA-filtered environments are desirable [43]. The average amount of Aspergillus conidia reported in the hospital air range from 0.6 to 21.1 CFU/m³ in various studies [23,26,30,32,36,44]. Air filtration measurements have been shown to decrease the number of conidia in the air down to less than 0.1 CFU/m³ have been reported [25,30,32].

Several potential sources for Aspergillus conidia in hospital air have been described, inadequate filtration of outside air by the air handling system being the most obvious one [42,46]. Dust with high concentrations of spores accumulates in air ducts and other places that are infrequently cleaned, and produces bursts of airborne Aspergillus spp. when disturbed [28]. Another well-documented source of bursts of Aspergillus conidia is vacuum cleaning [23], even when HEPA-filtered exhausts are used on the vacuum cleaner [42]. Furthermore, sudden air movements e.g. when opening a door to a room, is discussed as a source of (micro-)bursts.

Due to its clinical relevance, most studies on Aspergillus spp. in hospital air have dealt with A. fumigatus. Less is known about other Aspergillus species such as Aspergillus flavus and Aspergillus terreus and other filamentous fungi. Air sampling studies have found lower spore concentrations for A. flavus, A. terreus and Aspergillus niger than for A. fumigatus in both outside and indoor (hospital) air [27,31,33,34]. In hospital air, Hospenthal et al. [36] detected similar levels of A. fumigatus and A. flavus conidia, while the average conidial count of other Aspergillus spp. was slightly higher. It may well be assumed that the route of entry in the hospital is similar for all Aspergillus spp.
Water

Recently, attention was drawn to the role of hospital water as a possible source of filamentous fungi. Reasons to investigate other sources of filamentous fungi, and in particular *A. fumigatus* as the most frequent species causing invasive disease in immunocompromised patients, are two-fold. Preventive measures that lower the concentration of airborne conidia by use of HEPA-filtration failed to reduce the risk of invasive filamentous fungal infections to zero, and gave rise to suspicion of other sources and routes of transmission. The second reason is the lack of genetic relatedness between environmental hospital strains and those causing invasive disease.

That (drinking) water harbours filamentous fungal organisms, including *A. fumigatus*, and possibly also their metabolites, is not new [47,48]. But the possible relation between fungal contamination and opportunistic filamentous fungal infections in immunocompromised patients is from recent years. Anaissie [49] recovered opportunistic fungal pathogens from sinks and showerheads in several hospitals in the USA. *A. terreus* and *A. niger* were cultured from the showerheads as well as *Fusarium* species. Furthermore, during a three-year sampling period, 32% of 358 water samples revealed filamentous fungi and 21% were positive for *Aspergillus* spp. Filamentous fungi were recovered from only 4% of water-related surfaces of which 2% were *Aspergillus* spp. *A. niger* was the most frequent species found, *A. fumigatus* was only found in 1% of all the samples taken [50]. A study conducted in Norway revealed that 94% of water samples taken inside the hospital harboured filamentous fungi. *A. fumigatus* was recovered from 49% of water samples taken from the taps in the pediatric bone marrow transplantation unit of this hospital [51]. Furthermore, the recovery of higher contamination levels in water taken from the intake reservoir (surface water), supplying the hospital with water, suggested that the source was located outside the hospital [52]. During a recent survey in the hematology ward of the University Medical Centre in Nijmegen, the Netherlands, we were not able to culture *Aspergillus* spp. from hospital water, nor from the intake reservoir consisting of ground water. It might well be that the kind of intake reservoir (ground water vs. surface water) influences the level of contamination of hospital water, thereby explaining the differences observed [53].

Quality of water used in procedures like hemodialysis has always been a matter of concern. A recent paper from Arvanitidou et al. [54] in which the water quality in 85 hemodialysis centres in Greece was investigated, a high percentage of fungal contamination was found. 81% of the water samples revealed filamentous fungi with *Penicillium* spp. (39%) as the most often isolated fungus, followed by *Aspergillus* spp. (31%, mainly *A. niger*). Previously, the same investigator showed that filamentous fungi were isolated from 95% of community and 76% of hospital water samples, with dermatophytes fungi (63%) being the most prevalent [55]. Similar observations have been made in hemodialysis centres in other countries, which also showed more or less fungal contamination of water and dialysate samples [56,57]. The forming of biofilms in water distribution systems or in pipe-work supplying automatic cleaners for medical equipment yielded various filamentous fungi and might be responsible for sudden increases in the contamination level of hospital water [58,59].

Other sources

Aspergillus species, and other filamentous fungi like zygomycetes, may enter the hospital with various foodsstuffs including biscuits, tea, herbs and spices, shellfish, and fruit [60-62]. Organic material such as the soil of potted ornamental plants, and flowers may also serve as sources of *Aspergillus* spp. [63]. Contamination of pepper with *Aspergillus* spp. has been reported frequently [64-66]. In a recent study not only *Aspergillus* spp., but also Mucorales were found to contaminate all tested pepper samples [60]. Ice-making machines, nebulizers and ultrasonic humidifiers can be contaminated by *Aspergillus* conidia [67,68]. A recent study from Verweij et al. [69] showed that both tobacco and marijuana are heavily contaminated with filamentous fungi including *A. fumigatus*. Furthermore, growth of *Aspergillus* on air filters and within fireproofing material that had been used in hospital construction, with subsequent release of conidia inside the hospital has been reported [35,70].

Routes of transmission

Air

The primary route of acquiring *Aspergillus* infection is probably by the inhalation of conidia. Conidia of the most commonly involved pathogenic *Aspergillus* spp., i.e. *A. fumigatus, A. flavus* and *A. terreus*, are relatively small, with sizes ranging from 2 to 5 micron [71]. Due to their small size, conidia will become deposited deep into the lung after inhalation. In most individuals, inhaled conidia will be cleared by the alveolar macrophages, without affecting the individuals health [72]. Immunocompromised patients, however, are extremely susceptible to local invasion of respiratory tissues by deposited conidia, resulting in invasive aspergillosis [2]. Most cases of (nosocomial) invasive aspergillosis present with pneumonia [30,73,74]. Therefore, it has been hypothesized that the inhalation of airborne *Aspergillus* conidia, either directly or through intermediate nasopharyngeal colonization [42,75,76], is a direct cause of pulmonary infection in immunocompromised patients.

Arguments in favor of the airborne transmission are provided by many studies. Reduced incidence rates of invasive aspergillosis have been reported in hospitals after moving from older hospital buildings to new facilities with modernized air handling systems [46,77,78]. Moreover, the installation of HEPA-filtered air units for the care of immunocompromised patients has been reported to result in a reduction of *Aspergillus* conidia concentrations in ambient air. This reduction in concentration of airborne conidia is paralleled by a decrease in the frequency of invasive infections but does not reduce it to zero [14,42,45,79]. These observations suggest that a correlation exists between the concentration of *Aspergillus* conidia in the hospital air and the risk of invasive disease, although in other studies this correlation remains unclear [33,36,44].

Many investigators have described outbreaks of nosocomial invasive aspergillosis. Some of these case clusters have been associated with construction and/or renovation activities in and around hospitals [41,46,79-88], others with malfunctioning or contamination of hospital ventilation or air filtration systems [84,89-91]. Although the reported outbreaks suggest that the concentration of *Aspergillus* conidia in air plays an important role in the development of invasive aspergillosis, these data should be interpreted carefully. With the relatively low frequency of invasive aspergillosis seen in many hospitals, even small changes in the number of cases may appear to be a cluster when in fact it is not. Besides, most studies have documented potential sources of increased
conidial concentrations in the air, such as construction activities and contamination of air handling systems, retrospectively, which may have resulted in (observer) biased conclusions. Moreover, there are often no data available on the baseline air concentrations of conidia in order to determine whether these case clusters were associated with increased exposure to airborne Aspergillus spp.

Only few studies have evaluated the relation between the airborne concentration of Aspergillus spp. and the risk of invasive aspergillosis directly, with conflicting results. One group of investigators conducted a prospective study in which monthly environmental cultures for Aspergillus spp. and surveillance for nosocomial invasive aspergillosis were performed during a six-year period [35]. An increase in the mean concentrations of A. fumigatus and A. flavus conidia from less than 0.2 to more than one conidia per cubic meter of air was accompanied by a fourfold increase in the incidence of invasive aspergillosis from 0.3% to 1.2% in immunocompromised patients (P=0.01). Yet, in another study the occurrence of six cases of invasive aspergillosis could not be linked to changes in the recovery of airborne Aspergillus spp. during a one-year period with weekly air sampling of the ward rooms and corridors [36]. Recently, a significant relationship between environmental fungal contamination in hematology wards and the incidence of invasive aspergillosis in a non-epidemic setting was shown, during a four-year period [45]. The presence of Aspergillus spp. directly indicated a risk of aspergillosis. Furthermore, the total count of unspecified fungal spores in air samples served indirectly as a good marker of a higher risk of invasive aspergillosis. Although most of these fungi were non-pathogenic, their detection shows a lack of effective filtration or cleaning and/or conditions favouring the settling of filamentous fungi, including Aspergillus spp. [37,45].

Many studies that have attempted to associate cases of invasive aspergillosis with the environmental recovery of Aspergillus spp. have used only speciation in their comparisons. Recently, however, several studies have applied molecular techniques to compare environmental and clinical isolates. Analysis of moderately repeated (MIR) sequences of A. fumigatus isolated from patients and the environment revealed that A. fumigatus isolates from two of six patients were identical to hospital environment strains [92]. Molecular typing of clinical and environmental isolates during an outbreak of invasive A. flavus infections yielded comparable results [81]. That is, restriction fragment length polymorphism (RFLP) analysis revealed similar patterns in isolates from one patient and the environmental source, whereas this pattern differed from those found in other cases of invasive aspergillosis. In another study, a PCR-based method was used for discrimination of clinical and environmental A. fumigatus isolates during a three-year period. Eight out of ten genotypes of A. fumigatus isolates from bone marrow transplant patients were also found in the hospital environment, whereas six were present in the unit itself [93]. These findings suggest a nosocomial origin of the A. fumigatus infection. However, other researchers have failed to detect genetic relatedness between patient and environmental isolates [25,27,94]. These conflicting results may be explained by the fact, that the genetic diversity among clinical and environmental isolates of A. fumigatus is extremely high [95,96]. Even within groups of isolates with the same geographical origin no clustering of isolates was observed. Furthermore, it has been shown that persistence of identical genotypes of A. fumigatus for more than six months may exist in the hospital environment [37,92]. The results of these studies indicate that extensive air sampling, as well as typing of all recovered isolates may be necessary to link cases of invasive aspergillosis with environmental recovery of Aspergillus spp.

Studies on the relation between airborne Aspergillus conidia and invasive aspergillosis have primarily focused on in-hospital exposure to Aspergillus conidia. However, since little is known on the incubation period of invasive aspergillosis, it may well be that patients become colonized outside the hospital, either before or after discharge, and that invasive aspergillosis is, at least partly, a community acquired disease. Arguments in favor of this hypothesis, comes from a study by Einsele et al. [97], who found that transplant patients underwent a bronchoalveolar lavage in a screening procedure at the time of the transplantation. Seven out of 134 patients tested positive in the PCR for Aspergillus and five of them developed invasive aspergillosis during their immunosuppressive treatment after the transplantation. None of the patients with a negative PCR result developed invasive aspergillosis. This study suggests that preventive measures should not be aimed at the hospital environment only.

Water

The discovery of hospital water as a source of A. fumigatus and other filamentous fungi may suggest a new route for the transmission of invasive filamentous fungal infections. It might well be that conidia originating from water, become aerosolized during activities like showering. Subsequently, patients may inhale the aerosols containing Aspergillus conidia and become infected. Alternatively, conidia may be present on walls and floors of the bathroom, being disturbed by air flow changes which occur during showering. Initial studies indicate that there is an increase in conidial counts in the air in patient bathrooms over the day after multiple shower periods [51]. Anaissie et al. [98] showed that a gradient exists between patient bathrooms and patient rooms and hallways, with higher numbers of aerosolized conidia in the bathrooms. Furthermore, conidial air counts of Aspergillus spp. were increased during showering, suggesting that aerosolization occurs during showering. The clinical significance of these findings as yet remains unclear.

To prove a wet route of transmission, molecular characterization of isolates recovered from water and patients is warranted. Preliminary results obtained by analysing various isolates by amplified fragment length polymorphism (AFLP) showed genotypic relatedness between clinical isolates recovered from three patients with isolates recovered from water [99]. Although preliminary, these results suggest that a wet route of transmission may exist. Besides this, anecdotal reports have linked invasive aspergillosis with aspiration of contaminated surface water in near-drowning patients [100,101].

Other routes of transmission

Although inhalation of airborne conidia is believed to be the primary route of acquiring Aspergillus infection, alternative modes of transmission may be present. Breeches in the skin and intravenous insertion sites, with or without the use of adhesive tapes and armboards, can serve as a port d’entrée by disrupting the outer barrier integrity [102-104]. Contamination of wounds or the immature, thin skin in very low birth weight infants, by direct inoculation or from the surrounding air, has also been reported [105-107]. Recently, a cluster of cutaneous
A. flavus infections in the neonatal intensive care unit was investigated by the use of molecular methods (a repetitive DNA probe). A. flavus strains of the same genotype were isolated from both neonates, as well as from the tape used to fasten their umbilical catheters and the place where the tape was stored [108]. Contamination of food with Aspergillus species can be an indirect source of airway or digestive tract colonization [109]. Invasive pulmonary aspergillosis was associated with the heavy usage of marijuana, as a solely risk factor, during the post-transplant period in two patients [110,111]. Investigation of a cluster of cutaneous infections due to A. niger in neutropenic patients in a bone marrow transplantation unit, revealed heavy environmental contamination with black mould in the ward kitchen adjacent to the unit [112]. Typing of the clinical and environmental isolates by random amplification of polymorphic DNA (RAPD) showed one of the patients being infected with a genotypically identical strain, that previously was repeatedly isolated from the kitchen. In another case, contaminated stockinet material was implicated as the source of infection. Potted plants in a unit for high-risk patients was associated with A. terreus infections in patients with hematological malignancies. Based on RAPD patterns, the isolates of four patients were identical to those cultured from the potted plants [113]. Another report recovered a dual-reservoir cooler-heater used in the operation room to be the source of the A. flavus strain isolated from the aortic prostheses of a heart surgery patient, thereby indicating a nosocomial origin [114]. Some anecdotal reports have documented outbreaks of invasive aspergillosis associated with contaminated fire proofing material [70], damp wood, potting soil and carpeting [115].

Conclusions

With the continuing increase in the number of severely immunocompromised patients, hospitals are faced with the growing problem of invasive aspergillosis and other opportunistic fungal infections. Since treatment of these infections are difficult and outcome is often fatal, preventive measures are of major importance in the control of invasive filamentous fungal infections. Therefore, full understanding of the epidemiology is crucial in the development of effective preventive strategies. Although transmission by air is probably the most important route of infection, other possible routes of transmission might exist and should be recognized and investigated. Molecular characterization and comparisons of fungal isolates recovered from patients and environment is needed and will surely contribute to our understanding of the epidemiology of these devastating infections. Exclusively studying the role of the hospital environment as a cause of invasive aspergillosis might not be enough, since patients might already be colonized with the fungus in their home environment, before entering the hospital. Despite developing invasive disease during their hospital stay when the host defences are diminished, invasive aspergillosis may not always be of “nosocomial origin”. Another unsolved issue is whether invasive aspergillosis can arise from chronic, low level, endemic airborne Aspergillus conidia or whether a large inoculum is required. Future research should be designed to further elucidate the many questions that remain on the role of the hospital environment in the development of invasive aspergillosis and other filamentous fungal infections in immunocompromised patients.

References


