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

Adilia Warris[1,2], Andreas Voss[2] & Paul E. Verweij[2]

Departments of [1]Pediatrics and [2]Medical Microbiology, University Medical Centre St. Radboud, Nijmegen, The Netherlands

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?

Resumen

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

Dirección para correspondencia:
Dr. Adilia Warris
UMC St. Radboud
Department of Pediatrics, 435
P.O. Box 9101
6500 HB Nijmegen
The Netherlands
Tel.: +31 24 361 4430
Fax: +31 24 361 6428
E-mail: A.Warris@ckskg.azn.nl

1130-1406/01/10.00 Euros

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/m^3) [24,31,33]. Extremely high concentrations of airborne *A. fumigatus* conidia, up to 10^6 conidia/m^3, 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]. Futhermore, 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/m^3 in protective isolation suites, and less than 0.1 CFU/m^3 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/m^3 in various studies [23,27,30,32,36,44,45]. While in rooms with HEPA-filtration counts below 0.1 CFU/m^3 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 dematiaceous 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 foodstuffs 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 DNA 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 from Einsele *et al.* [97], were bone marrow 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 were 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 the 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 stockinette 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 prosthesis 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

1. Bodey GP. The emergence of fungi as major hospital pathogens. J Hosp Infect 1988; 11: 411-426.
2. Denning DW. Invasive aspergillosis. Clin Infect Dis 1998; 26: 781-805.
3. Groll AH, Shah PM, Mentzel C, Schneider M, Just-Nuebling G, Huebner K. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. J Infect 1996; 33: 23-32.
4. Sulahian A, Boutboul F, Ribaud P, Leblanc T, Lacroix C, Derouin F. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. Cancer 2001; 91: 311-318.
5. Yeghen T, Kibbler CC, Prentice HG, *et al.* Management of invasive aspergillosis in hematology patients: a review of 87 consecutive cases at a single institution. Clin Infect Dis 2000; 31: 859-868.
6. Hovi L, Saarinen-Pihkala UM, Vettenranta K, Saxen H. Invasive fungal infections in pediatric bone marrow transplant recipients: single center experience of 10 years. Bone Marrow Transplant 2000; 26: 999-1004.
7. Paterson DL, Singh N. Invasive aspergillosis in transplant recipients. Medicine 1999; 78:123-38.
8. Winkelstein JA, Marino MC, Johnston RB, *et al.* Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine 2000; 79: 155-169.
9. Mylonakis E, Paliou M, Sax PE, Skolnik PR, Baron MJ, Rich JD. Central nervous system aspergillosis in patients with human immunodeficiency virus infection: report of 6 cases and review. Medicine 2000; 79: 269-280.
10. Gerson SL, Talbot GH, Hurwitz S, Strom BL, Lusk EJ, Cassileth PA. Prolonged granulocytopenia: the major risk factor for invasive aspergillosis in patients with acute leukemia. Ann Intern Med 1984; 100: 345-351.
11. Mehrad B, Paciocco G, Martinez FJ, Ojo TC, Iannettoni MD, Lynch JP III. Spectrum of *Aspergillus* infection in lung transplant recipients: case series and review of the literature. Chest 2001; 119: 169-175.
12. Denning DW. Therapeutic outcome in invasive aspergillosis. Clin Infect Dis 1996; 23: 608-615.
13. Denning DW, Stevens DA. Antifungal and surgical treatment of invasive aspergillosis: a review of 2,121 published cases. Rev Infect Dis 1990; 12: 1147-1201.
14. Pannuti CS, Gingrich RD, Pfaller MA, Wenzel RP. Nosocomial pneumonia in adult patients undergoing bone marrow transplantation: a 9-year study. J Clin Oncol 1991; 9: 77-84.
15. Walsh TJ. Invasive aspergillosis in patients with neoplastic diseases. Sem Resp Infect 1990; 5: 111-22.
16. Weinberger M, Elattar I, Marshall D, *et al.* Patterns of infection in patients with aplastic anemia and the emergence of *Aspergillus* as a major cause of death. Medicine 1992; 71: 24-43.
17. Hiemenz JW, Walsh TJ. Lipid formulations of amphotericin B: recent progress and future directions. Clin Infect Dis 1996; 22: S133-S144.
18. Mills W, Chopra R, Linch DC, Goldstone AH. Liposomal amphotericin B in the treatment of fungal infections in neutropenic patients: a single-centre experience of 133 episodes in 116 patients. Br J Haematol 1994; 86: 754-760.
19. Oppenheim BA, Herbrecht R, Kusne S. The safety and efficacy of amphotericin B colloidal dispersion in the treatment of invasive mycoses. Clin Infect Dis 1995; 21: 1145-1153.
20. Stevens DA. Overview of amphotericin B colloidal dispersion (Amphocil). J Infect 1994; 28: S45-S49.
21. Patterson TF, Kirckpatrick WR, White M, *et al.* Invasive aspergillosis: Disease spectrum, treatment practices, and outcomes. Medicine 2000; 79: 250-60.

22. Walsh TJ, Dixon DM. Nosocomial aspergillosis: environmental microbiology, hospital epidemiology, diagnosis and treatment. Eur J Epidemiol 1989; 5: 131-142.
23. Anderson K, Morris G, Kennedy H, *et al.* Aspergillosis in immunocompromised paediatric patients: association with building hygiene, design, and indoor air. Thorax 1996; 51: 256-261.
24. Jones BL, Cookson JT. Natural atmospheric microbial conditions in a typical suburban region. Appl Environ Microbiol 1983; 45: 919-934.
25. Leenders A, van Belkum A, Janssen S, *et al.* Molecular epidemiology of apparent outbreak of invasive aspergillosis in a hematology ward. J Clin Microbiol 1996; 34: 345-351.
26. Mullins J, Hutcheson PS, Slavin RG. *Aspergillus fumigatus* spore concentration in outside air: Cardiff and St. Louis compared. Clin Allergy 1984; 14: 351-354.
27. Rath PM, Ansorg R. Value of environmental sampling and molecular typing of aspergilli to assess nosocomial sources of aspergillosis. J Hosp Infect 1997; 37: 47-53.
28. Rhame FS. Prevention of nosocomial aspergillosis. J Hosp Infect 1991; 18: 466-472.
29. Rose HD, Hirsch SR. Filtering hospital air decreases A*spergillus* spore counts. Am Rev Respir Dis 1979; 119: 511-513.
30. Sherertz RJ, Belani A, Kramer BS, *et al.* Impact of air filtration on nosocomial *Aspergillus* infections. Am J Med 1987; 83: 709-718.
31. Solomon WR, Burge HP, Boise JR. Airborne *Aspergillus fumigatus* levels outside and within a large clinical center. J Allergy Clin Immunol 1978; 62: 56-60.
32. Withington S, Chambers ST, Beard ME, *et al.* Invasive aspergillosis in severely neutropenic patients over 18 years: impact of intranasal amphotericin B and HEPA filtration. J Hosp Infect 1998; 38: 11-18.
33. Goodley JM, Clayton YM, Hay RJ. Environmental sampling for aspergilli during building construction on a hospital site. J Hosp Infect 1994; 26: 27-35.
34. Noble WC, Clayton YM. Fungi in the air of hospital wards. J Gen Microbiol 1963; 32: 397-402.
35. Arnow PM, Sadigh M, Costas C, Weil D, Chudy R. Endemic and epidemic aspergillosis associated with in-hospital replication of *Aspergillus* organisms. J Infect Dis 1991; 1: 998-1002.
36. Hospenthal DR, Kwon-Chung KJ, Bennett JE. Concentration of airborne *Aspergillus* compared to the incidence of invasive aspergillosis: lack of correlation. Med Mycol 1998; 36: 165-168.
37. Leenders ACAP, van Belkum A, Behrendt M, Luijendijk A, Verbrugh HA. Density and molecular epidemiology of *Aspergillus* in air and relationship to outbreaks of *Aspergillus* infection. J Clin Microbiol 1999; 37: 1752-1757.
38. Li DW, Kendrick B. A year-round study on functional relationships of airborne fungi with meteorological factors. Int J Biometeorol 1995; 39: 74-80.
39. Patterson JE, Zidouh A, Miniter P, Andriole VT, Patterson TF. Hospital epidemiologic surveillance for invasive aspergillosis: patient demographics and the utility of antigen detection. Infect Control Hosp Epidemiol 1997; 18: 104-108.
40. Clark CS, Rylander R, Larsson L. Levels of gram-negative bacteria, *Aspergillus fumigatus*, dust, and endotoxin at compost plants. Appl Environ Microbiol 1983; 45: 1501-1505.
41. Streifel AJ, Lauer JL, Vesley D, Juni B, Rhame FS. *Aspergillus fumigatus* and other thermotolerant fungi generated by hospital building demolition. Appl Environ Microbiol 1983; 46: 375-378.
42. Rhame FS, Streifel AJ, Kersey JH, Jr., McGlave PB. Extrinsic risk factors for pneumonia in the patient at high risk of infection. Am J Med 1984; 76: 42-52.
43. Morris G, Kokki MH, Anderson K, Richardson MD. Sampling of *Aspergillus* spores in air. J Hosp Infect 2000; 44: 81-92.
44. Cornet M, Levy V, Fleury L, *et al.* Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against *Aspergillus* airborne contamination during hospital renovation. Infect Control Hosp 1999; 20: 508-513.
45. Alberti C, Bouakline A, Ribaud P, *et al.* Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients. J Hosp Infect 2001; 48: 198-206.
46. Sarubbi FA, Kopf HB, Wilson MB, McGinnis MR, Rutala WA. Increased recovery of *Aspergillus flavus* from respiratory specimens during hospital construction. Am Rev Respir Dis 1982; 125: 33-38.
47. Nagy LA, Olson BH. The occurrence of filamentous fungi in drinking water distribution systems. Can J Microbiol 1982; 28: 667-671.
48. Paterson RRM, Kelley J, Gallagher M. Natural occurrence of aflatoxins and *Aspergillus flavus* (link) in water. Lett Applied Microbiol 1997; 25: 435-436.
49. Anaissie EJ. Emerging fungal infections: don't drink the water. San Diego, California: Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy 1998; J-93 and S-147.
50. Anaissie EJ, Stratton SL, Summerbell RC, Rex JH, Walsh TJ. Pathogenic *Aspergillus* species recovered from a hospital water system: a three-year prospective study. Toronto, Canada: Abstracts of the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy 2000; J-1321.
51. Warris A, Gaustad P, Meis JFGM, Voss A, Verweij PE, Abrahamsen TG. Recovery of filamentous fungi from water in a paediatric bone marrow transplantation unit. J Hosp Infect 2001; 47: 143-148.
52. Warris A. Hospital water as a source of filamentous fungi: A new route of transmission? Abstract. Rev Iberoam Micol 2000; 17: S112.
53. Warris A, Gaustad P, Voss A, Abrahamsen TG, Verweij PE. Contamination of Hospital Water with *A. fumigatus* Is Influenced by the Natural Reservoir. Chicago, Illinois: Abstracts of the 41th Interscience Conference on Antimicrobial Agents and Chemotherapy 2001; 255.
54. Arvanitidou M, Spaia S, Velegraki A, *et al.* High level of recovery of fungi from water and dialysate in haemodialysis units. J Hosp Infect 2000; 45: 225-230.
55. Arvanitidou M, Kanellou K, Constantinides TC, *et al.* The occurence of fungi in hospital and community potable water. Lett Appl Microbiol 1999; 29: 81-84.
56. Bambauer R, Schauer M, Jung WK, *et al.* Contamination of dialysis water and dialysate. A survey of 30 centers. ASAIO J 1994; 40: 1012-1016.
57. Klein E, Pass T, Harding GB, *et al.* Microbial and endotoxin contamination in water and dialysates in the central United States. Artif Organs 1990; 14: 85-94.
58. Doggett MS. Characterization of fungal biofilms within a municipal water distribution system. Appl Environ Microbiol 2000; 66: 1249-1251.
59. Phillips G, McEwan H, Mckay I, Crowe G, McBeath J. Black pigmented fungi in the water pipe-work supplying endoscope washer disinfectors. J Hosp Infect 1998; 40: 250-251.
60. Bouakline A, Lacroix C, Roux N, Gangneux JP, Derouin F. Fungal contamination of food in hematology units. J Clin Microbiol 2000; 38: 4272-4273.
61. Sallenave-Namont C, Pouchus YF, Robiou du Pont T, *et al.* Toxigenic saprophytic fungi in marine shellfish farming areas. Mycopathologia 2000; 149: 21-25.
62. Schwab AH, Harpestad AD, Swartzentruber A, *et al.* Microbiological quality of some spices and herbs in retail markets. Appl Environ Microbiol 1982; 44: 627-630.
63. Staib F. Ecological and epidemiological aspects of aspergilli pathogenic for man and animal in Berlin (West). Zentralbl Bakteriol Mikrobiol Hyg [A] 1984; 257: 240-245.
64. De Bock R, Gyssens I, Peetermans M, Nolard N. *Aspergillus* in pepper. Lancet 1989; 2: 331-332.
65. Eccles NK, Scott GM. *Aspergillus* in pepper. Lancet 1992; 339: 618.
66. Vargas S, Hughes WT, Giannini M. *Aspergillus* in pepper. Lancet 1990; 336: 881.
67. Alvarez-Fernandez JA, Quirce S, Calleja JL, *et al.* Hypersensitivity pneumonitis due to an ultrasonic humidifier. Allergy 1998; 53: 210-212.
68. Kibbler CC. Epidemiology of fungal disease. In: Kibbler CC, Mackenzie DWR, Odds FC (Eds.) Principles and practice of clinical mycology. Chicester, John Wiley & Sons Ltd. 1996: 13-21.
69. Verweij PE, Kerremans JJ, Voss A, Meis JF. Fungal contamination of tobacco and marijuana. JAMA 2000; 284: 2875.
70. Aisner J, Schimpff SC, Bennet JE, Young VM, Wiernik PH. *Aspergillus* infections in cancer patients. JAMA 1976; 235: 411-412.
71. Bennett JE. *Aspergillus* species. In: Mandell GL, Bennet JE, and Dolin R (Eds). Mandell, Douglas and Bennett's principles and practice of infectious diseases. New York, Churchill Livingstone 1995: 2306-2311.
72. Schaffner A, Douglas H, Braude A. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus*: observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. J Clin Invest 1982; 69: 617-631.
73. Fridkin SK, Harvis WR. Epidemiology of nosocomial fungal infections. Clin Microbiol Rev 1996; 9: 499-511.
74. Young RC, Bennet JE, Vogel CL, Carbone PP, DeVita VT. Aspergillosis. The spectrum of the disease in 98 patients. Medicine 1970; 49: 147-173.
75. Aisner J, Murillo J, Schimpff SC, Steere AC. Invasive aspergillosis in acute leukemia: correlation with nose cultures and antibiotic use. Ann Intern Med 1979; 90: 4-9.
76. Martino P, Raccah R, Gentile G, Venditti M, Girmenia C, Mandelli F. *Aspergillus* colonization of the nose and pulmonary aspergillosis in neutropenic patients: a retrospective study. Haematologica 1989; 74: 263-265.
77. Rose HD. Mechanical control of hospital ventilation and *Aspergillus* infections. Am Rev Respir Dis 1972; 105: 306-307.
78. Rosen PP, Sternberg SS. Decreased frequency of aspergillosis and mucormycosis. N Engl J Med 1976; 295: 1319-1320.
79. Oren I, Haddad N, Finkelstein R, Rowe JM. Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters. Am J Hematol 2001; 66: 257-62.
80. Arnow PM, Andersen RL, Mainous PD, Smith EJ. Pulmonary aspergillosis during hospital renovation. Am Rev Respir Dis 1978; 118: 49-53.
81. Buffington J, Reporter R, Lasker BA, *et al.* Investigation of an epidemic of invasive aspergillosis: utility of molecular typing with the use of random amplified polymorphic DNA probes. Pediatr Infect Dis J 1994; 13: 386-393.
82. Burwen DR, Lasker BA, Rao N, Durry E, Padhye AA, Jarvis WR. Invasive aspergillosis outbreak on a hematology-oncology ward. Infect Control Hosp Epidemiol 2001; 22: 45-48.
83. Krasinski K, Holzman RS, Hanna B, Greco MA, Graff M, Bhogal M. Nosocomial fungal infection during hospital renovation. Infect Control 1985; 6: 278-282.

84. Lentino LR, Rosenkranz MA, Michaels JA, Kurup VP, Rose HD, Rytel MW. Nosocomial aspergillosis. A retrospective review of airborne disease secondary to road construction and contaminated air conditioners. Am J Epidemiol 1982; 116: 430-437.
85. Loo VG, Bertrand C, Dixon C, *et al.* Control of construction-associated nosocomial aspergillosis in an antiquated hematology unit. Infect Control Hosp Epidemiol 1996; 17: 360-364.
86. Lueg EA, Ballagh RH, Forte V. Analysis of the recent cluster of invasive fungal sinusitis at the Toronto hospital for sick children. J Otolaryngol 1996; 25: 366-370.
87. Tabarra KF, Jabarti AA. Hospital construction-associated outbreak of ocular aspergillosis after cataract surgery. Opthalmology 1998; 105: 522-526.
88. Weems JJ, Davis BJ, Tablan OC, Kaufman L, Martone WJ. Construction activity: an independent risk factor for invasive aspergillosis and zygomycosis in patients with hematologic malignancy. Infect Control 1987; 8: 71-75.
89. Kyriakides GK, Zinneman HH, Hall WH, *et al.* Immunologic monitoring and aspergillosis in renal transplant patients. Am J Surg 1976; 131: 246-252.
90. Mahoney DH, Jr., Steuber CP, Starling KA, Barrett FF, Goldberg J, Fernbach DJ. An outbreak of aspergillosis in children with acute leukemia. J Pediatr 1979; 95: 70-72.
91. Ruutu P, Valtonen V, Tiitanen L, *et al.* An outbreak of invasive aspergillosis in a haematologic unit. Scand J Infect Dis 1987; 19: 347-351.
92. Girardin H, Sarfati J, Traoré F, Dupouy Camet J, Derouin F, Latgé J. Molecular epidemiology of nosocomial invasive aspergillosis. J Clin Microbiol 1994; 32: 684-690.
93. Radford SA, Johnson EM, Leeming JP, *et al.* Molecular epidemiological study of *Aspergillus fumigatus* in a bone marrow transplantation unit by PCR amplification of ribosomal intergenic spacer sequences. J Clin Microbiol 1998; 36: 1294-1299.
94. Mellado E, Diaz-Guerra TM, Cuenca-Estrella M, *et al.* Characterization of a possible nosocomial aspergillosis outbreak. Clin Microbiol Infect 2000; 6: 543-548.
95. Chazalet V, Debeaupuis JP, Sarfati J, *et al.* Molecular typing of environmental and patient isolates of *Aspergillus fumigatus* from various hospital settings. J Clin Microbiol 1998; 36: 1494-1500.
96. Debeaupuis J, Sarfati J, Chazalet V, Latgé JP. Genetic diversity among clinical and environmental isolates of *Aspergillus fumigatus*. Infect Immun 1997; 65: 3080-3085.
97. Einsele H, Quabeck K, Muller KD, *et al.* Prediction of invasive pulmonary aspergillosis from colonisation of lower respiratory tract before marrow transplantation. Lancet 1998; 352: 1443.
98. Anaissie EJ, Stratton SL, Summerbell RC, Monson TP, Rex JH, Walsh TJ. *Aspergillus* species aerosols in hospitals: showering as a potential mode of exposure. San Francisco, California: Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy 1999; J-1908.
99. Verweij PE, Warris A, Gaustad P, Klaassen CHW, Meis JFG. Molecular typing of *Aspergillus fumigatus* recovered from water and patients. Chicago, Illinois: Abstracts of the 41th Interscience Conference on Antimicrobial Agents and Chemotherapy 2001.
100. ter Maaten JC, Golding RP, Strack van Schijndel RJ, Thijs LG. Disseminated aspergillosis after near-drowning. Neth J Med 1995; 47: 21-4.
101. Mizukane R, Sawatari K, Araki J, *et al.* Invasive pulmonary aspergillosis caused by aspiration of polluted water after nearly drowning. Kansenshogaku Zasshi 1996; 70: 1181-1185.
102. van Burik JAH, Colven R, Spach DH. Cutaneous aspergillosis. J Clin Microbiol 1998; 36: 3115-3121.
103. Isaac M. Cutaneous aspergillosis. Dermatol Clin 1996; 14: 137-140.
104. Walsh TJ. Editorial response: primary cutaneous aspergillosis – an emerging infection among immunocompromised patients. Clin Infect Dis 1998; 27: 453-457.
105. Gettleman LK, Shetty AK, Prober CG. Posttraumatic invasive *Aspergillus fumigatus* wound infection. Ped Infect Dis J 1999; 18: 745-747.
106. Groll AH, Jaeger G, Allendorf A, Herrmann G, Schloesser R, von Loewenich V. Invasive pulmonary aspergillosis in a critically ill neonate: case report and review of invasive aspergillosis during the first 3 months of life. Clin Infect Dis 1998; 27: 437-452.
107. Walmsley S, Devi S, King S, Schneider R, Richardson S, Ford-Jones L. Invasive *Aspergillus* infections in a pediatric hospital: a ten-year review. Ped Infect Dis J 1993; 12: 673-682.
108. James MJ, Lasker BA, McNeil MM, Shelton M, Warnock DW, Reiss E. Use of a repetitive DNA probe to type clinical and environmental isolates of *Aspergillus flavus* from a cluster of cutaneous infections in a neonatal intensive care unit. J Clin Microbiol 2000; 38: 3612-3618.
109. Sarfati J, Jensen HE, Latgé JP. Route of infections in bovine aspergillosis. J Med Vet Mycol 1996; 34: 379-383.
110. Hamadeh R, Ardehali A, Locksley RM, York MK. Fatal aspergillosis associated with smoking contaminated marijuana, in a marrow transplant recipient. Chest 1988; 94: 432-433.
111. Marks WH, Florence L, Lieberman J, *et al.* Successfully treated invasive pulmonary aspergillosis associated with smoking marijuana in a renal transplant recipient. Transplantation 1996; 61: 1771-1774.
112. Loudon KW, Coke AP, Burnie JP, Shaw AJ, Oppenheim BA, Morris CQ. Kitchens as a source of *Aspergillus niger* infection. J Hosp Infect 1996; 32: 191-198.
113. Lass-Florl C, Rath P, Niederwieser D, *et al. Aspergillus terreus* infections in haematological malignancies: molecular epidemiology suggests association with in-hospital plants. J Hosp Infect 2000; 46: 31-35.
114. Diaz-Guerra TM, Mellado E, Cuenca-Estrella M, Gaztelurrutia L, Navarro JI, Tudela JL. Genetic similarity among one *Aspergillus flavus* strain isolated from a patient who underwent heart surgery and two environmental strains obtained from the operating room. J Clin Microbiol 2000; 38: 2419-22.
115. Gerson SL, Parker P, Jacobs MR, Creger R, Lazarus HM. Aspergillosis due to carpet contamination. Infect Control Hosp Epidemiol 1994; 15: 221-223.