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Hospital water originating from ground water is a source of molds other than *Aspergillus* species

Summary

Background: The incidence of invasive mold infections among immunocompromised patients has considerably increased during the last decades despite the introduction of preventive measures, including air filtration. Thus, besides the air additional sources of infection in the hospital environment may play a role in the acquisition of *Aspergillus* and other pathogenic molds.

Methods: We investigated water (hot shower water, cold tap water, originating from ground water), air, and environmental sources (sinks and shower basin before and after running of water, exit of compressed oxygen and dry surfaces) in the hematology unit of our hospital for contamination with pathogenic molds.

Results: Regarding the concentration of molds, no significant difference was found between cold and warm water samples. After showering, the concentration of airborne molds increased significantly to a median of 3.5 CFU/m^3 air ($p<0.05$). The highest numbers of molds were found in swabs from the water basin sinks after flushing with water. In the shower baths, the amount of molds found before and after showering did not differ significantly. Identification of the fungi yielded molds other than *Aspergillus* in all cases.

Conclusion: No major contamination with *Aspergillus spp.* was detected in water sources, supporting the idea that sources of ground water harbor lower numbers of *Aspergillus spp.* than drinking water originating from surface water. Our study contributes to the hypothesis that molds are secondarily airborne when water is run in showers or sinks. It appears prudent to clean wet surfaces before water use in order to minimize the risk of mold infection in immunocompromised patients.
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Introduction

Opportunistic invasive mold infections, in particular those caused by *Aspergillus* species, are a serious threat for immunocompromised patients [1]. Patients with hematological malignancies and stem cell transplantation recipients are especially at risk [2,3,4]. Infections with pathogenic molds are thought to be mainly acquired by inhalation of mold spores that are ubiquitous in the environment. The incidence of invasive mold infections has considerably increased during the last decades despite the introduction of preventive measures like the use of high-efficiency particulate air (HEPA) filters and laminar airflow systems [4,5].

The increasing incidence of invasive mold infections, in particular invasive aspergillosis, has led to the assumption that besides the air additional sources of infection in the hospital environment may play a role in the acquisition of *Aspergillus* and other pathogenic molds. The hospital water system has drawn special attention as a possible reservoir of pathogenic molds since hospital water is known to be a resource for nosocomial infections like legionellosis and diseases caused by gram-negative bacilli, especially *Pseudomonas aeruginosa*, and mycobacteria [6,7,8,9].

Aspergillus and other pathogenic molds have been detected in environmental water sources and, in recent surveys, also in hospital water systems [10,11,12,13,14, 15,16,17,18]. Airborne molds were found in significantly higher concentrations in areas of major water use, such as patient shower rooms [19], suggesting that aerosolization of waterborne molds may occur after or during water use. Cleaning of patient shower facilities can reduce the mean air concentrations of molds, including *Aspergillus spp.* [20]. In one recent study performed in Norway, molecular typing of water and clinical isolates of *Aspergillus fumigatus* confirmed hospital water as the source of invasive aspergillosis in pediatric patients with hematological malignancies [18]. Strains recovered from water samples were genetically different from those recovered from air [18].

In contrast to the data presented above, Warris et al. did not recover any pathogenic molds including *Aspergillus spp.* from water samples collected at the Nijmegen University Medical Center, The Netherlands [17]. The authors speculate that the supply of drinking water from surface water rather than from ground water may be contaminated with molds. While the drinking water supplying the Nijmegen hospital originates from ground water [17], the hospital in the study conducted in Norway [18] is supplied by surface water.

In the adult hematology unit of our University Hospital, HEPA filtration of the air of a 38-bed ward was newly installed in April 2001 in an attempt to reduce the increasing incidence of invasive aspergillosis. However, the incidence of probable and proven cases of invasive aspergillosis in hematological patients did not decrease during the following years despite successful elimination of airborne molds. Although the drinking water supply of our hospital originates from ground water we assumed that waterborne molds may be a source of the clinical infections. Therefore, we investigated potential water and environmental sources of *Aspergillus spp*. and molds in general in the hematology ward, including shower and tap water samples, air measurements and surface swabs.

Materials and Methods

Environment

The study was conducted on an adult hematology ward at the University Hospital of Ulm, Germany. The ward contains 38 beds in 19 rooms and is equipped with a central HEPA filtration system. All HEPA filters are routinely monitored at 6 month intervals and maintained to ensure a high rate of air exchange. The hospital is served by the municipal water supply of the city of Ulm. Municipal water originates from ground water sampled in a depth of up to 18 meters. The ground water is only treated with low doses of chlorine (final concentration of chlorine dioxide in the drinking water: 0.03 mg/l) and is not further processed. Municipal water entering the hospital is regularly monitored according to water industry standards. In the hospital, the water is transported in standpipes and ringlines consisting of galvanised steel and dead pipe ends are avoided. Ten randomly selected rooms of the ward were included in the study. Each two-bed room contains a separated water basin and a shower. The sampling sites within each room are depicted in Figure 1.

Water samples

One-liter cold tap water samples and one-liter warm water from the shower were collected in sterile glass bottles. Regarding shower water, the shower was run until the water reached a temperature of at least 30 °C and then a one-liter sample was immediately taken. Within one hour after sampling, water samples were passed through a sterile 0.45-µm cellulose acetate filter by use of a filtration apparatus (Millipore, Eschborn, Germany) within a laminar airflow cabinet. With use of sterile forceps, the filters were placed directly on Sabouraud dextrose agar plates (Heipha, Heidelberg, Germany). Plates were incubated at 30 °C for 14 days.

Surface samples

Surface samples were taken with a sterile swab transport system (Hain Diagnostika, Nehren, Germany) with transport medium or with 56-mm Sabouraud dextrose agar contact plates containing 50 mg/l chloramphenicol (Heipha, Heidelberg, Germany). Sampling sites included the sink of the wash basin (sampling site 1 in Figure 1) and of the shower bath (sampling site 2 in Figure 1) before and after water sampling (swab), the shower basin (sampling site 3) before and after water sampling (contact plate), exit of decompressed air in the wall (sampling site 4, swab), and surfaces of the telephone of bed 1 (sampling site 5), the bed tables of both beds (sampling sites 6 and 7), the windowsill (sampling site 8), and the floor in front of bed 1 (sampling site 9, all contact plates, see Figure 1). After sampling, the swabs were inoculated onto Sabouraud dextrose agar plates as described above. Contact plates were directly transferred into the 30 °C incubator.

Air samples

Samples of air (1,000 liter each) were collected by use of a MAS-100 air sampler (Merck, Darmstadt, Germany). Sampling sites included the shower bath before and after five minutes of showering, two sites on the ward hall, exterior air on the balcony of the ward, and the air obtained from the outlets of the compressed oxygen supply provided in each patient room. The air was directly collected onto Sabouraud dextrose agar plates and the plates were incubated as described above.

Identification of molds

Aspergillus species were identified by standard methods including macroscopic and microscopic morphology at different temperatures (30 °C, 36 °C and 42 °C) [21]. All other molds were not identified to the genus and species level and are referred to as "molds other than *Aspergillus spp.*". Colony counts were enumerated as colony-forming units (CFU) per liter, for water samples, or per cubic meter, for air samples.

Statistical analysis

Significances of differences were analyzed by the Wilcoxon signed rank test using the software StatView version 5.0.1.

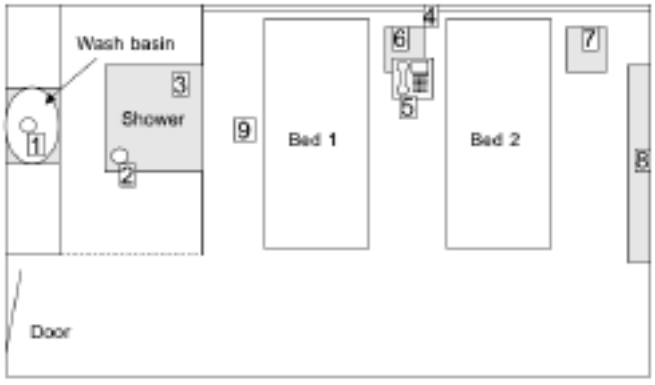

Figure 1: Overview over the sampling sites.

Results

Water sampling

Molds were found in 9 of 10 cold water samples with a median concentration of 2 CFU/l (range of positive samples 1 to 22 CFU/l) and in 7 of 10 warm shower

water samples with a median concentration of 2 CFU/l (range of positive samples 1 to 20 CFU/l, see also Table 1). Regarding the concentration of molds, no significant difference was found between cold and warm water samples. All molds found in the water samples were identified as molds other than *Aspergillus spp.*

Air sampling

Air samples within the patient shower bath before showering revealed molds in nine out of ten rooms with a median concentration of 1.5 CFU/m^3 air (range of positive samples 1 to 4 CFU/m^3). After showering, the concentration of molds increased significantly to a median of 3.5 CFU/m^3 air (range of positive samples 1 to 13 CFU/m^3) ($p < 0.05$). No molds were found in the samples of compressed oxygen (Table 1). In none of the air samples obtained in the ward hall or rooms *Aspergillus spp.* was found. Air sampling of the outside air from the balcony revealed 60 CFU/m3 molds including 3 CFU/m^3 *Aspergillus fumigatus.*

Surface sampling

Molds were found in two out of ten swabs taken at the water basin sinks before water sampling and in four after water sampling, reaching no significant difference (Table 1). Highest numbers of molds were found in the swabs from the water basin sinks taken after water sampling, i.e. after flushing of the sink with water (Table 1). In the shower baths, molds were found on five swabs and eight contact plates out of the ten rooms, but results of swabs and contact plates sampled before and after showering did not differ significantly (Table 1). Only minute numbers of molds (1 CFU) were found at the outlet of compressed oxygen in three out of ten rooms while surface samples from the telephone, bed tables, windowsill, and floor revealed molds in three to nine of the ten rooms in moderate numbers (Table 1). In none of the surface samples *Aspergillus spp.* was found.

Table 1: Results of water, air, and surface sampling for molds.

Sampling sites (see also Figure 1)	Positive for molds	Median	Range of positive samples
Cold tap water (n=10)	9	2 CFU/l	1–20 CFU/l
Warm shower water (n=10)	7	2 CFU/l	1–22 CFU/l
Outside air (balcony) (n=1)	1	60 CFU/m^3	–
Ward hall air (n=2)	0	0 CFU/m^3	–
Shower bath air before showering (n=10)	9	1,5 CFU/m^3	1–4 CFU/m^3
Shower bath air after showering (n=10)	9	3,5 CFU/m^3	1–13 CFU/m^3*
Compressed air (n=10)	0	0 CFU/m^3	–
1: Surface water basin sink before water sampling (n=10)	2	0 CFU	3–35 CFU
1: Surface water basin sink after water sampling (n=10)	4	0 CFU	3–110 CFU
2: Surface shower basin sink before showering (n=10)	5	1 CFU	2–23 CFU
2: Surface shower basin sink after showering (n=10)	6	1,5 CFU	1–21 CFU
3: Surface shower bath before showering (n=10)	8	3,5 CFU	1–11 CFU
3: Surface shower bath after showering (n=10)	5	1,5 CFU	3–16 CFU
4: Surface exit compressed air (n=10)	3	0 CFU	1 CFU
5: Surface telephone bed 1 (n=10)	7	1,5 CFU	1–15 CFU
6: Surface bed table bed 1 (n=10)	5	0,5 CFU	1–105 CFU
7: Surface bed table bed 2 (n=10)	3	0 CFU	1–8 CFU
8: Surface windowsill (n=10)	3	0 CFU	1–6 CFU
9: Surface floor (n=10)	9	1 CFU	1–6 CFU

*significant difference before and after showering, p<0.05.

Discussion

Despite the lack of controlled studies, HEPA filtration of the air has been regarded as an efficient and recommended measure for prevention of invasive mold infections like aspergillosis in hematology units [22]. However, installation of air filtration did not decrease the incidence of invasive mold infections in other institutions [2,4] as well as in our own hospital. Therefore, other sources of molds causing infections have been searched. Hospital water systems have been assumed to be a reservoir for pathogenic molds, in particular *Aspergillus spp.*, causing invasive fungal infections in immunocompromised patients, especially in hematology units. Indeed, in several recent studies, presence of *Aspergillus spp.* and other molds in hospital water samples has been demonstrated [10,11,12,15,16,17,19,23]. In addition, hospital drinking water as a source of clinical infection has been confirmed in recent studies [10,18]. It has been speculated that the origin of the drinking water, i.e. ground or surface water, might be essential regarding contamination of the drinking water with molds: Drinking water that originates from ground water seems to be less contaminated with molds than water that originates from surface water [17]. Our study confirms this assumption, since the University Hospital of Ulm is served with drinking water originating from ground water. We did not find any *Aspergillus spp.* in cold or warm water samples taken from ten rooms in our hematology unit. In addition, the median and maximal concentrations of molds in water samples from our hospital were much lower (2 and 22 CFU/l, respectively) than those reported by Warris et al. from the Oslo University Hospital that is served with drinking water originating from surface water [16].

Regarding airborne molds, we confirmed the findings of Anaissie et al. concerning *Aspergillus spp.* [10], detecting higher concentrations of molds in the air in the shower bath than in the ward hall (Table 1). Although the concentrations of molds in the drinking water in our hospital were low, a significant increase in the concentration of airborne molds after showering was noted in the shower bath. Thus, not only *Aspergillus spp.* but also other molds seem to be aerosolized during showering. Anaissie et al showed a significant decrease of aerosolized mold con-

centrations by cleaning the shower walls before running the water [20]. These findings in conjunction with our data suggest that molds are secondarily airborne from wet surfaces during water use. Regarding the clinical significance of these findings a species identification of all molds would have been very helpful but was, unfortunately, not provided in our study.

Surfaces of the water and the shower basins as well as the water and shower basin sinks were contaminated with moderate amounts of molds in 20–80 % of samples (Table 1). However, there was no statistically significant difference between the numbers found on surfaces before and after flushing the tap water or showering, respectively. Investigation of siphons would be valuable to further determine the reservoir of the molds, however, in this preliminary study siphons were not included due to technical reasons. On dry surfaces, molds were found in all rooms in low to moderate numbers.

While searching for alternative sources of molds causing clinical infections we speculated that compressed oxygen given to the patients via a nose mask may also be contaminated with molds. However, all compressed oxygen samples were negative for molds although minute amounts of molds were detected in three out of ten outlets of the compressed oxygen. Thus, the use compressed oxygen does not seem to be a risk for the patients regarding acquisition of invasive mold infections.

In conclusion, the drinking water in our hospital serves as a reservoir for molds other than *Aspergillus spp.* This source may constitute an additional risk factor for the acquisition of invasive fungal infections in severely immunocompromised patients. Further studies, including identification of all molds with respect to pathogenic species, are necessary to elucidate the significance of the source and primary treatment of the drinking water with regard to the risk of transmission of pathogenic molds including *Aspergillus* species.

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