

Keywords

*Aspergillus fumigatus**Fusarium solani*

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Growth behavior and persistence of *Aspergillus fumigatus* and *Fusarium solani* in drinking water

Summary

Background: Recently published data suggest the hypothesis that pathogenic moulds in hospital water distribution systems might be a source of invasive fungal infections (e.g., aspergillosis, fusariosis) in immunocompromised patients. Our study aimed to investigate the growth behavior and persistence of two of the most important fungi, *Aspergillus fumigatus* and *Fusarium solani*, in drinking water.

Method: Drinking water samples were inoculated with reference strains and incubated at a water temperature of 5 °C, 25 °C, 37 °C, and 55 °C over a period of four years.

Results: Both fungal strains persisted for more than 12 months at 5 °C and more than 48 months (after a transient growth phase) at 25 °C, but were rapidly inactivated at higher temperatures. *F. solani* rapidly produced extracellular matrix floating in the drinking water, whereas *A. fumigatus* only could be found at the interface water/air.

Conclusion: We conclude that based on the growth behaviour and the ability to reproduce within biofilms *F. solani* must be regarded as a potential colonizer of hospital water distribution systems. In contrast, data do not support such an association for *A. fumigatus*. The clinical impact of this supposed source of invasive fungal infections should be evaluated in future studies.

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Nidulans, etc.) as well as a large number of representatives of other mould genera (*Fusarium*, *Scedosporium/Pseudallescheria*, *Exophiala*, etc.) are known to be potential pathogens [1,2,3].

Because of the ubiquitous nature of moulds, such as *A. fumigatus* in the outdoor air, nosocomial aspergillosis has been viewed to date as being primarily an airborne infection [2]. Accordingly, the main prophylactic measures taken to prevent exposure included ensuring that immunosuppressed patients were provided with a supply of air that was free, or harboured only a reduced count, of conidia.

That paradigm which held sway hitherto was recently questioned by Anaissie et al. who postulated that there was a link between the presence of *Fusarium solani* and *Aspergillus fumigatus* in the hospital water distribution system and the occurrence of invasive fusarioses [4] or aspergilloses [5,6].

The aim of the present pilot study was to gain insights into the growth patterns and persistence of these two species of moulds in drinking water as a function of the water temperature.

Materials and Methods

The study was conducted with the following reference strains: *Aspergillus fumigatus* (CBS 192.62); *Fusarium solani* (CBS 340 C.070).

The primary culture of strains was made on 2 % malt extract agar (2 % MEA).

Using an inoculation eyelet, material was carefully harvested from the conidia

Introduction

Exogenous mould infections pose a considerable threat to immunosuppressed patients. Apart from *Aspergillus fumigatus*, which in epidemiologically terms is, no doubt, the prime candidate, other *Aspergillus* species (*A. flavus*, *A. niger*, *A. terreus*, *A.*

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and added to drinking water samples from the hospital's water distribution system to ensure a baseline concentration of $0.4 \pm 0.1 \times 10^3$ cfu/ml. The drinking water was obtained from the cold water tap of the mycology laboratory after allowing it to run until it reached a steady temperature and flaming. The water quality conformed with all requirements of the German Drinking Water Regulation 2001. After mixing the sample for 10 minutes in a shaker, aliquots of the mixture were immediately transferred to 100 ml Schott bottles. The aliquots were then stored at 5 °C, 25 °C, 37 °C and 55 °C. The water samples were initially inspected on a weekly basis, then as from the third month on a monthly basis and finally after the second year on a six-monthly basis.

After mixing the samples for two minutes in a shaker, aliquots of 1.0 and 0.1 ml, and if necessary other dilution stages, were directly plated onto 2 % MEA and incubated for seven days at 25 °C. Tests were conducted in duplicate.

In view of the low fluctuation rates seen in the values calculated in parallel for the various dilution stages, the arithmetic mean value was calculated and illustrated in the figures.

Results

Neither for *Aspergillus fumigatus* nor *Fusarium solani* was any growth observed in the number of colony forming units in the drinking water samples at either 5 °C, 37 °C or 55 °C (Figure 1 and 2). Conversely, rapid multiplication of *A. fumigatus* was seen at an incubation temperature of 25 °C (onset after around two months, maximum after about seven months by almost 2 log levels up to 2.8×10^4 cfu/ml). Multiplication was less pronounced for *F. solani* (onset after around three weeks, maximum after about four months up to 1.9×10^3 cfu/ml).

For details of persistence, please consult Table 1. At 55 °C neither mould species was detectable any longer after only one week, but at 37 °C both species persisted somewhat longer. At a water temperature of 25 °C, both mould species could now be detected after a, in the meantime, 48-month observation period, albeit at a concentration that was more than one log level lower than the baseline concentration. Regression dynamics was less pro-

Table 1: *in vitro* persistence of *A. fumigatus* and *F. solani* in drinking water as a function of the water temperature (observation duration to date: 48 months).

Water temperature	<i>A. fumigatus</i>	<i>F. solani</i>
5° C	12 months	32 months
25° C	> 48 months	> 48 months
37° C	10 weeks	2 weeks
55° C	n.d. after 1 week	n.d. after 1 week

n.d. = non detectable

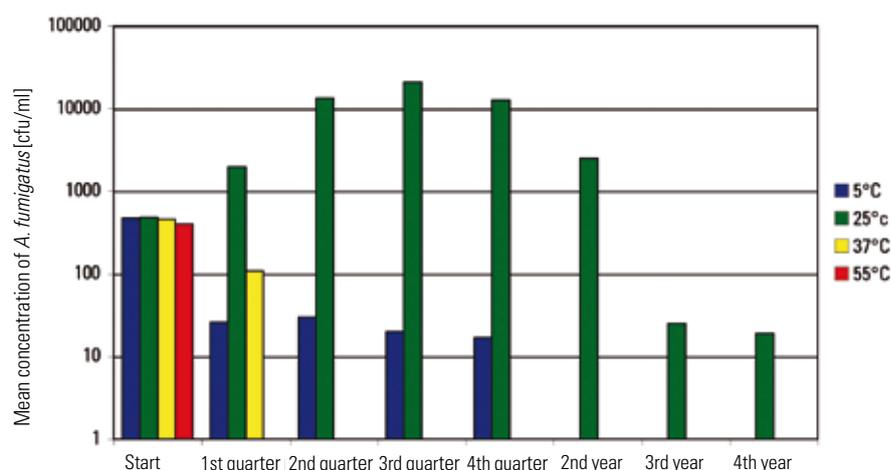


Figure 1: Persistence of *Aspergillus fumigatus* (CBS 192.62) in drinking water over a four-year observation period at different water temperatures.

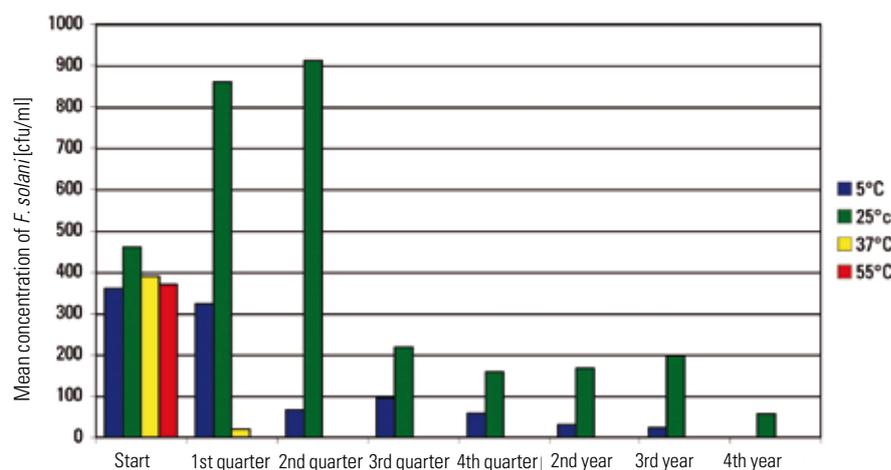


Figure 2: Persistence of *Fusarium solani* (CBS 340 C.070) in drinking water over a four-year observation period at different water temperatures.

nounced for *F. solani* than in the case of *A. fumigatus*. At 5 °C, we noted persistence of both species over a period of at least one year despite there being no growth.

Discussion

Our investigations show that under experimental conditions both mould species multiplied and persisted for at least four years in the drinking water samples despite the limited supply of nutrients.

In terms of growth patterns, marked differences were noted between the two species. Whereas *F. solani* gave rise to a mucus / flocculent matrix after an incubation period of less than one week at 25 °C, which was still evident after > 48 months, *A. fumigatus* was noted primarily at the water / air interfaces or on the inside of receptacles.

From culture on solid nutrient media, the conidia of *F. solani* are known to form mucus structures (formation of conidia in "false" mucus heads or mucus layers above the substrate [7,8].

Contrary to our expectations, at 37 °C neither growth nor long-term persistence was noted for either species in drinking water. The optimal temperature for linear growth is 27–31 °C for *F. solani*, but at 37 °C good growth is also evidenced depending on the substrate [7]. *A. fumigatus* grows within a very broad temperature range of 12–57°C, with optimum growth at 37–43°C [7].

At a water temperature of 55°C, there was no longer any evidence of active mould growth at the time of the first follow-up inspection after just one week, and at least in the case of *F. solani* that conformed to our expectations. These findings confirm our (so far unpublished) observations suggesting that there is hardly any evidence of moulds in hot drinking water samples taken from the hospital's water distribution system.

To what extent the results of these in vitro tests can be extrapolated to the conditions prevailing within the water distribution system remains unclear. For *A. fumigatus* we conclude that it is unlikely that it grows within the actual distribution system and that growth appears to be possible only at sites where the water comes into contact with air (e.g. in taps, shower heads, toilet flushing systems, etc.).

That conclusion is also corroborated by the findings of a study conducted by Warris et al. [9], who detected low concentrations (mean value: 1.9 cfu/500 ml) of *A. fumigatus* in 49 % of cold water samples taken from an Oslo hospital water distribution system. However, the incoming supply system had a higher load outside the hospital (85 % of samples were positive, mean value: 3.1 cfu/ml), making the authors conclude that the source of contamination was located outside the hospital. On the other hand, in shower water samples taken from the hot water supply (60 °C) *A. fumigatus* was seen only in 5.6 % of samples and at a lower concentration (mean value: 1.0 cfu/500 ml) [9]. Freije [10], too, deemed the evidence implicating the water supply as the source of transmission of *Aspergillus* to be "too incomplete" as to justify active monitoring

Conversely, the possibility of *F. solani* multiplying in the water distribution system cannot be discounted, especially since this species is able to grow well even under conditions of reduced oxygen partial pressure and CO₂ concentrations of up to 20 % are tolerated without any significant impairment of growth [7]. The ability to adhere to, and form biofilm on, PVC is well documented – even in flowing water [11].

However, the incidence of growth of *Fusarium* species in water distribution systems and the clinical implications of its detection in biofilm remain unclear. By comparing clinical and environmental isolates (water and air samples, swabs from siphons, shower heads and perlaters, etc.) of *Fusarium* species using molecular biology typing methods (RFLP, RAPD, IR-PCR) Anaissie et al. [1] postulated that hospital water distribution systems were a potential source of fusarioses in immunosuppressed patients.

Raad et al. [12], on the other hand, claimed that only two of the 20 patient isolates from that study were environmental isolates and that these corresponded only to those from siphons and, based on the findings of their own study, they questioned this transmission route but without ruling out that it could apply in isolated cases.

O'Donnell et al. [13], based on extensive genotyping of clinical and environmental isolates of the *Fusarium-oxysporum* complexes, however, corroborate the first mentioned hypothesis.

In summary, it can be concluded that the mould species investigated in the present study demonstrate a surprising ability to persist in drinking water and that, because of their growth patterns in hospital water distribution systems, biofilm-associated moulds such as *F. solani* pose a potential risk when caring for immunosuppressed patients. Further studies are needed to quantify the magnitude of that risk.

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Conflict of Interest

The authors declare that there is no conflict of interest as understood by the International Committee of Medical Journal Editors.

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